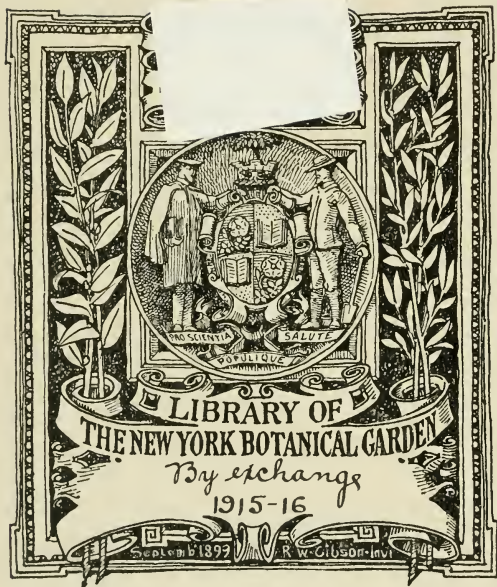




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ERRATA

- Page 20, last line, "ammonium sulphate" should read "ammonium carbonate."
- Page 22, legend under figure 16 should read "Diagram showing the number of corn plants up and dry matter produced in 21 days on College loam with sodium sulphate, sodium carbonate, and sodium chlorid," etc.
- Page 23, legend under figure 18 should read "Diagram showing the number of wheat plants up and dry matter produced in 16 days on Greenville loam with ammonium carbonate, sodium carbonate, and potassium carbonate," etc.
- Page 59, "*Dipsacus fullonum*" should read "*Dipsacus fullonum*."
- Page 63, "*Capsula bursa pastoris*" should read "*Capsella bursa pastoris*."
- Pages 65, 66, 67, "*Helianthus diversicatus*" should read "*Helianthus divaricatus*."
- Page 174, line 17 from bottom, "32°" should read "30°."
- Page 175, Table II, last column, last line, "60.0" should read "67.0."
- Page 189, line 13 from bottom, "form" should read "from."
- Page 191, line 17 from bottom, "Fusarium Wollenw." should read "Fusarium hyperoxysporum Wollenw."
- Page 210, Plate XVII, "figure 1" should read "figure 4," "figures 2, 3, 4" should read "figures 1, 2, 3."
- Page 271, line 22, "weight of the kernels" should read "weight of the extracted kernels."
- Page 272, line 8, "It was normal" should read "It was not normal."
- Page 279, footnote b, "Rabbit 651" should read "Rabbit 951."
- Page 291, "*Panax quinquefolia*" should read "*Panax quinquefolium*."
- Page 334, line 11 from bottom should read "thermoelements in this section may be observed."
- Page 694, line 7, "2 N 2/5" should read "N 2/5."
- Page 700, line 14, "N/5" should read "N 2/5."
- Page 752, footnote, line 2 from bottom, should read "For 100 c. c. synthetic solution take 1 c. c. of M/5 magnesium sulphate, 1 c. c. asparagin M/5, and 5 c. c. of each of the other solutions, and add to 88 c. c. water. Steam on three successive days."
- Page 780, Plate LVI, figure 2, B, "0.1 per cent" should read "0.01 per cent."
- Page 782, "*Pinus murrayana* Oreg. Com." should read "*Pinus contorta* Loud."
- Page 911, line 13 from bottom should read "and a humid soil after the removal of lime and magnesia,"
- Page 912, line 10 from bottom should read "10 gm. of dry soil after the removal of lime and magnesia,"
- Plate LXVI, "Fig. 2" should read "Fig. 1."
- Page 986, last line, "also" should read "next to."
- Page 987, first footnote, "eighth" should read "seventh."
- Page 1016, line 16, "comparing them" should read "comparable."
- Page 1023, Table VII, first column, "12.15 a. m." should read "12.15 p. m."
- Page 1036, line 4, "spore" should read "pore."
- Page 1063, line 3, "4.0065 feet" should read "4.0056 feet."
- Page 1071, figure 8 and tenth line from bottom of page, " $C=3.078L^{1.022}$ " should read " $C=3.078L^{0.022}$."
- Page 1073, line 17, "4.0058 feet" should read "4.0086 feet."
- Page 1081, Table VIII, "4.0058-foot notch" should read "4.0086-foot notch."
- Page 1083, bottom of page, " $H\left(2.5-\frac{0.0195}{S_{0.75}}\right)$ " should read " $H\left(2.5-\frac{0.0195}{S_{0.75}}\right)$."
- Page 1095, Table XIV, under "Head, 1 foot," ninth column, tenth line, "4.52" should read "4.53."
- Page 1112, Literature cited, "Forschheimer" should read "Forchheimer."
- Page 1117, legend under figure 1, end of line 6, "spring" should read "fall."
- Page 1187, "22.9" should read "12.9."

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ANNOUNCEMENT OF WEEKLY PUBLICATION

The past two years have witnessed the partial realization of a plan, long favored by officers of this Department and of the State Agricultural Experiment Stations, for centralized publication of some of the results of scientific research upon agricultural problems.

With the more general appreciation of the economic necessity for further researches in all sciences allied to agriculture and the recognition of the suitability of the Journal of Agricultural Research for recording new data of fundamental significance to agriculture, the papers awaiting publication in this Journal have increased to a degree that warrants more frequent issuance. Beginning with Volume V, therefore, a number of the Journal of Agricultural Research will appear each week.

D. F. HOUSTON,
Secretary of Agriculture

Washington, D. C.,
September 2, 1915

JOURNAL OF AGRICULTURAL RESEARCH

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NO. 1

EFFECT OF ALKALI SALTS IN SOILS ON THE GERMINATION AND GROWTH OF CROPS

By FRANK S. HARRIS,¹

Professor of Agronomy, Utah Agricultural Experiment Station

INTRODUCTION

In arid regions the soil is likely to contain an accumulation of soluble salts in such quantities that the growth of vegetation is hindered. Indeed, in many sections the type of vegetation is determined almost entirely by the alkali content of the soil. Every grade may be found, from the soil containing so much soluble salt that no vegetation whatever will grow to the soil containing scarcely sufficient soluble material for the needs of plants.

In the western part of the United States there are millions of acres of land of each alkali type. The worst of these lands need not be considered at present for agricultural purposes, but there are vast areas just on the border line. If everything is favorable, they produce profitable crops; but during the average year crops are a failure. If a permanent agriculture is to be established on these soils, it will be necessary to increase greatly our knowledge of methods of handling them.

A large part of the unsettled land of the West contains more or less alkali. Chemical analysis of the soil can easily be made and the alkali content determined; where the alkali content is very high, the land is not suited to agriculture; where it is low, the alkali can not be considered an interfering factor. It is the soil containing a medium amount that causes the difficulty. Many projects that were condemned when an analysis of the soil was made have proved later to be fertile agricultural tracts. On the other hand, lands whose salt content was thought to be sufficiently low for crop production have later been abandoned. There are not sufficient exact experimental data available to make it

¹ The author wishes to acknowledge his indebtedness to his assistants, Messrs. Howard J. Maughan, George Stewart, and A. F. Bracken, for their faithful and intelligent efforts in conducting certain parts of the work; to Mr. R. M. Madsen, Miss Alma Esplin, and Mr. N. I. Butt for their care in making many laborious computations; and to a number of other faithful assistants who helped in conducting the experiments.

possible in all cases to determine how well crops will grow in a soil of known alkali content.

In view of the great practical importance of the subject as well as its scientific interest, considerably more information should be gathered on the relation of alkali in soils to crops. The limits of endurance of each crop for each salt in the different kinds of soil should be fixed with much greater exactness.

It was in response to this need that the work reported in this article was undertaken.

REVIEW OF THE LITERATURE

The effect on plants of the salts classed as alkali has been the subject of much investigation, but the greater part of this work has been done in solution cultures rather than in the soil. By using water cultures an attempt has been made to limit the great number of factors that exist in the soil, where some of the salts are neutralized and others are absorbed. The work of Loew (16),¹ Kearney (12-14), Harter (7, 14), Cameron (5, 13), Breazeale (1-2, 5), Dorsey (6), Osterhout (20-21), True (26), McCool (18), and others in this country and numerous workers in Europe has added many facts to our knowledge of the action of single salts and balanced solutions on plants grown in water cultures. These workers have shown the great toxicity of salts like magnesium when used alone in a water culture and how this toxicity may be reduced by the presence of other elements.

The facts obtained in these experiments have increased our knowledge of plant physiology and the fundamental nature of alkali; but conclusions drawn from them should not be too definitely applied to the action of alkali as it is found in the soil.

For example, in solution cultures the salts of magnesium when present alone are very toxic, while if added to a normal soil they are no more toxic than a number of other salts. Again, Kearney and Cameron (13) concluded from their work with solutions that "the toxic effect of injurious salts is due very much more to the influence of the cations (derived from the basic radicle) than to the anions (furnished by the acid radicle)." This may be true for solution cultures, but it certainly does not always hold for salts added to soils, as the results in the present paper will show.

It is desirable, therefore, in studying the effect of soil alkali on plants to use soil as a medium in which to grow the plants, even though it is somewhat difficult to watch all the factors involved.

In 1876 Toutphoeus (9), and Henri Vilmorin (9) about the same time, published results of experiments showing that chemical fertilizers when added to the soil in too large quantities inhibit the germination of seeds.

¹ Reference is made by number to "Literature cited," p. 52-53.

Nessler in 1877 (9) stated that 0.5 per cent of cooking salt (sodium chlorid) injured the germination of rape, clover, and hemp, and that wheat withstands this solution, but is injured by a 1 per cent solution.

Hilgard was a pioneer in the study of alkali soils and as early as 1877 began publishing results on his investigations in California. From that time to the present his contributions, together with those of Loughridge, his associate, have constantly enriched the literature. Their results are contained in numerous publications of the California Agricultural Experiment Station and were well summarized by Hilgard in 1906 (11).

An excellent review of the work done on alkali in the United States up to 1905 is also given by Dorsey (6). A large proportion of the work on alkali in this country has consisted of the analysis of soils for the determination of the presence of various alkali salts.

A number of workers, however, have investigated the amounts of the different salts necessary to inhibit crop growth. Hilgard (10) and Loughridge (17) made numerous studies of the alkali content of California soils and the limits of concentration of the various salts at which cultivated and native plants cease to grow.

Buffum (3), Slosson (23), and Knight and Slosson (15) in Wyoming carried on many experiments on the effect of alkali on the germination of seeds and growth of crops. From their results they concluded that there is a regular decrease in the germination of seeds as the osmotic pressure increases; and there is no apparent difference between sodium or potassium, or between the sulphate and chlorid of the same or different salts. It will be noted that this conclusion is not borne out by the data contained in the present paper.

Headden (8), working with sugar beets, found that varieties differed in their resistance to alkali. He also determined the effect of sodium carbonate, sodium sulphate, and magnesium sulphate on the germination of sugar-beet seed. He concluded that—

The best seed germinated freely in soil containing as much as 0.10 per cent of sodium carbonate but the plants were attacked by as much as 0.05 per cent and it is doubtful whether any of them can survive when there is as much as 0.10 per cent of this salt present in the soil. Sodium sulphate affects the germination to a much less degree, even when it is equal to 0.90 per cent of the air-dried soil, but it is injurious when present in larger quantities. When both sodium carbonate and sodium sulphate are present in equal quantities, the action of the carbonate, or black alkali, is only slightly or not at all mitigated. Magnesium sulphate retards, but does not prevent germination when present in quantities equal to 1 per cent of the air-dried soil.

Stewart (25) made germination tests of a number of crops in soil to which different quantities of alkali salts had been added. He found sodium carbonate to be the most injurious of the alkalis with most crops. However, with white clover and red clover white alkali proved as injurious as the black. In their resistance to alkali the cereals stood in the following order: Barley, rye, wheat, and oats, barley being the most

resistant. He found that 0.50 per cent of either carbonate or chlorid was fatal to germination in almost all cases.

Hicks (9) found that—

Muriate of potash and sodium nitrate used as fertilizers in strengths of 1 per cent or more are very detrimental to the germination of seeds, whether applied directly or mixed with the soil; that the chief injury to germination from chemical fertilizers is inflicted upon the young sprouts after they leave the seed coat and before they emerge from the soil, while the seeds themselves are injured only slightly or not at all.

Shaw (22) after a great many tests was led to the conclusion that wherever the chlorid content of soil approached 0.2 per cent beet culture was unsuccessful.

Kearney (12) listed crops most likely to succeed in alkali of various concentrations, as follows: Excessive alkali (above 1.5 per cent), native and foreign saltbush and salt grasses; very strong alkali (1.0 to 1.5 per cent), date palm and pomegranate bushes; strong alkali (0.8 to 1 per cent), sugar beets, western wheat-grass, awnless brome-grass, and tall meadow oat-grass; medium strong alkali (0.6 to 0.8 per cent), meadow fescue, Italian rye-grass, slender wheat-grass, foxtail millet, rape, kale, sorgo, and barley for hay; medium alkali (0.4 to 0.6 per cent), redtop, timothy, orchard grass, cotton, asparagus, wheat for hay, oats for hay, rye, and barley; weak alkali (0.0 to 0.4 per cent), wheat for grain, emmer for grain, oats for grain, kafir, milo, proso millet, alfalfa, field peas, vetches, horse beans, and sweet clover.

Miyake (19), working on the effect of the chlorids, nitrates, sulphates, and carbonates of sodium, calcium, magnesium, and potassium on rice, found that the antagonistic action of individual salts was in part overcome when the salts were combined.

PRELIMINARY STUDIES

RESULTS IN 1912

The study of soil alkali in its relation to the growth of plants was begun by the Utah Experiment Station in 1912. The first tests were made in glass tumblers which held about 200 gm. of soil. The soil used was loam from the Greenville (Utah) Experimental Farm. The chemical and physical analyses of this soil are given in Tables VIII and IX.

The crops were New Zealand wheat (*Triticum aestivum*) and sugar beets (*Beta vulgaris*), 10 seeds being planted in each glass. Each sugar-beet seed, or ball, contains more than one germ; hence, more plants were usually obtained than the number of seeds planted.

The salts were added from stock solutions and were thoroughly mixed with the soil two or three days before the seeds were planted, July 28. The sugar beets were harvested on August 5, and the wheat on August 10. The plants that had come up were counted and their height and dry weight determined. The results are given in Tables I, II, and III.

TABLE I.—Percentage of germination of wheat and sugar beets in soil containing sodium chlorid, sodium carbonate, sodium sulphate, and magnesium sulphate in different concentrations. Salts added in solution

Concentration of salts (p. p. m. of dry soil).	Percentage of germination.							
	Wheat.				Sugar beets.			
	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.
None.	90	90	90	90	123	123	123	123
100.	60	100	80	80	90	50	100	80
500.	90	70	40	90	150	40	70	100
1,000.	60	70	80	80	170	90	110	120
2,000.	40	70	60	90	130	120	120	160
3,000.	0	50	50	60	20	100	200	110
4,000.	0	50	70	70	0	130	210	180
5,000.	0	80	60	50	0	150	250	70
6,000.	0	70	60	90	0	90	190	120
7,000.	0	30	80	60	0	20	210	210
8,000.	0	40	40	80	0	0	150	240
9,000.	0	30	70	60	0	0	100	180
10,000.	0	0	40	70	0	0	110	210

TABLE II.—Average height (in centimeters) of wheat and sugar-beet plants raised in soil containing alkali salts in various concentrations

Concentration of salts (p. p. m. of dry soil).	Average height of plants.							
	Wheat.				Sugar beets.			
	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.
None.	24	24	24	24	7	7	7	7
100.	24	24	27	25	7	7	7	7
500.	24	24	26	21	7	7	7	7
1,000.	17	23	26	27	7	7	7	7
2,000.	8	23	27	25	3	7	7	7
3,000.	22	23	27	3	7	7	5
4,000.	22	19	25	6	6	5
5,000.	22	21	25	6	6	6
6,000.	20	19	26	4	6	6
7,000.	10	12	22	3	5	7
8,000.	4	10	22	6	5
9,000.	3	5	23	4	5
10,000.	7	26	4	5

TABLE III.—Quantity of dry matter (in grams) produced by wheat and sugar-beet plants raised in soil containing alkali salts in various concentrations

Concentration of salts (p. p. m. of dry soil).	Dry matter.							
	Wheat.				Sugar beets.			
	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.	Sodium chlorid.	Sodium carbonate.	Sodium sulphate.	Magnesium sulphate.
None.....	0.131	0.131	0.131	0.131	0.020	0.020	0.020	0.020
100.....	.095	.142	.100	.118	.016	.101	.019	.013
500.....	.185	.082	.070	.117	.032	.007	.012	.017
1,000.....	.050	.110	.103	.147	.034	.015	.020	.018
2,000.....	.034	.117	.121	.114	.017	.023	.005	.027
3,000.....		.098	.075	.114	.005	.023	.033	.029
4,000.....		.080	.078	.108		.026	.039	.031
5,000.....		.119	.080	.073		.026	.048	.013
6,000.....		.090	.088	.144		.013	.035	.023
7,000.....		.039	.052	.060		.002	.035	.037
8,000.....		.034	.036	.129			.025	.038
9,000.....		.010	.048	.094			.015	.033
10,000.....			.029	.126			.015	.035

While the data in the tables are somewhat irregular on account of the comparatively small number of plants used, a few facts come out rather clearly. Probably the most conspicuous of these is the relatively high toxicity of sodium chlorid (NaCl) in Greenville soil when compared with other salts.

Two thousand p. p. m., or 0.2 per cent, marked the limit of growth for wheat, while three thousand p. p. m. was the limit for sugar beets. There was germination and growth with considerably more sodium carbonate (Na_2CO_3) than sodium chlorid, although the carbonate dissolved the organic matter from the soil, producing a very bad physical condition. Magnesium sulphate (MgSO_4) was only slightly toxic at a concentration of 1 per cent of the soil, while sodium sulphate (Na_2SO_4) was more toxic, but produced fair crops where 1 per cent was present. The percentage of germination, the height of plants, and the dry weight all correspond in showing where the growth began to be retarded by salt.

In order to determine the effect of the percentage of soil moisture on the toxicity of alkali, tests were made with soils having 12.5, 15, 17.5, 20, 22.5, 25, 27.5, and 30 per cent of water on the dry basis. At the one extreme the soil was about as dry as plants would grow in, while at the other it was completely saturated. The soil used was Greenville loam, and the seed planted was New Zealand wheat. The methods were the same as those already described, with 10 seeds in each glass.

The seeds were planted on August 16 and the plants harvested on September 27. The results are shown in Table IV.

TABLE IV.—*Effect of soil moisture on the toxicity of sodium carbonate on wheat plants*

NUMBER OF SEEDS GERMINATED IN EACH GLASS

Concentration of sodium carbonate (p. p. m. of dry soil).	Percentage of soil moisture.							
	12.5	15	17.5	20	22.5	25	27.5	30
4,000.....	8	9	9	9	9	8	7	9
5,000.....	8	8	8	8	7	6	6	10
6,000.....	2	9	9	7	5	9	7	8
7,000.....	4	3	8	5	6	5	3	5
8,000.....	1	8	7	6	4	3	3	4
9,000.....	5	5	5	3	3	1	1
10,000.....	3	5	5	4	3	1
11,000.....	1	1	2	4	3	3	2	1

AVERAGE HEIGHT OF PLANTS (CENTIMETERS) .

4,000.....	14	20	23	29	25	27	25	26
5,000.....	12	19	24	23	24	25	24	27
6,000.....	10	20	21	21	19	23	22	26
7,000.....	7	6	20	10	14	21	10	12
8,000.....	1	8	8	8	8	13	16	7
9,000.....	5	5	4	4	5	7	4
10,000.....	0	1	3	3	8	7	1
11,000.....	1	1	2	3	2	4	3	4

DRY MATTER PRODUCED PER GLASS (GRAMS)

4,000.....	0.090	0.136	0.147	0.216	0.197	0.166	0.155	0.196
5,000.....	.072	.123	.125	.131	.131	.120	.112	.202
6,000.....	.028	.131	.145	.113	.097	.145	.137	.154
7,000.....	.026	.018	.139	.046	.090	.085	.050	.078
8,000.....	0	.040	.055	.051	.048	.055	.074	.052
9,000.....	0	.019	.020	.024	.019	.035	.043	.021
10,000.....	0	0	.011	.008	.018	.065	.036	.028
11,000.....	.001	.001	.010	.019	.013	.014	.016	.025

From Table IV it is seen that the number of seeds germinating, the average height of plants, and the dry matter produced all decrease with the increased concentration of the alkali. The plants appear able to endure alkali better with a fair supply of moisture in the soil than where the soil is dry. This may be due to the fact that the soil solution is diluted by the water. Where the soil moisture was as low as 12.5 per cent, growth practically ceased at 7,000 p. p. m. of sodium carbonate, but in the wetter soils there was growth with as high a concentration as 11,000 p. p. m.

RESULTS IN 1913

On account of the inability to use a large number of seeds in glass tumblers, germination tests were made in tin plates in which 100 seeds could be used. An equivalent of 150 gm. of dry soil was placed in each tin plate and the necessary quantity of dry salt added. The salt was well mixed into the soil, which was made up to about 20 per cent of moisture. The seeds were planted and the pans covered with glass to prevent the escape of moisture. The number of seeds germinating was determined every day for three weeks. The results are summarized in Table V.

TABLE V.—Percentage of germination of seeds of New Zealand wheat which germinated in 21 days in Greenville soil containing various alkali salts. Salts added dry

Concentration of salt (p. p. m. of dry soil).	Percentage of germination.					
	Sodium chlorid.	Sodium car- bonate.	Sodium sul- phate.	Magnesium sulphate.	Equal parts of sodium chlorid, so- dium carbon- ate, sodium sulphate, and magnesium sulphate.	Equal parts of sodium chlorid, so- dium carbon- ate, sodium sulphate, and magnesium sulphate + 1 per cent of calcium sul- phate.
None.....	92	92	92	92	92	92
2,000.....	65	84	100	89	88	86
4,000.....	6	92	91	89	86	83
6,000.....	2	81	69	90	63	47
8,000.....	0	88	53	91	13	13
10,000.....	0	99	12	86	8	0
12,000.....	0	62	14	92	0	0
14,000.....	0	21	17	85	0	0
16,000.....	0	7	2	79	0	0
18,000.....	0	4	0	88	0	0
20,000.....	0	0	1	83	0	0

On examining Table V it is seen that sodium chlorid was by far the most toxic of the alkali salts and magnesium sulphate the least. The data given can not be taken as final, since all of the salts were not entirely dissolved and white salts could be seen scattered throughout the soil. The low harmfulness of sodium carbonate was probably due in part to the fact that it is not so readily soluble as the other salts when applied dry.

The mixed salts were more harmful than any single salt, with the exception of sodium chlorid, and it is probable that the harmfulness of the mixed salts was due largely to the sodium chlorid.

Since there was such a great difference in the effects of the various salts, a second experiment was made to determine more exactly the critical point of concentration. The results of this test are summarized in Table VI.

TABLE VI.—Percentage of germination of New Zealand wheat in soil containing alkali salts in different quantities. Salts added dry

Sodium chlorid.		Sodium carbonate.		Sodium sulphate.		Magnesium sulphate.		Equal parts of sodium chlorid, sodium carbonate, sodium sulphate, and magnesium sulphate.		Equal parts of sodium chlorid, sodium carbonate, sodium sulphate, and magnesium sulphate + 1 per cent of calcium sulphate.	
P. p. m.	Seed germination.	P. p. m.	Seed germination.	P. p. m.	Seed germination.	P. p. m.	Seed germination.	P. p. m.	Seed germination.	P. p. m.	Seed germination.
None.	P. ct. 92	None.	P. ct. 92	None.	P. ct. 92	None.	P. ct. 92	None.	P. ct. 92	None.	P. ct. 92
800	81	10,000	81	2,000	80	12,000	86	4,000	77	1,000	77
1,600	82	11,100	64	4,000	83	14,000	79	5,000	78	2,000	79
2,400	76	12,200	66	6,000	85	16,000	75	6,000	54	3,000	79
3,200	50	13,300	32	8,000	79	18,000	82	7,000	51	4,000	79
4,000	13	14,400	50	10,000	69	20,000	81	8,000	76	5,000	73
4,800	6	15,500	36	12,000	43	22,000	78	9,000	19	6,000	75
5,600	7	16,600	38	14,000	20	24,000	87	10,000	12	7,000	68
6,400	0	17,700	23	16,000	16	26,000	66	11,000	6	8,000	46
7,200	0	18,800	13	18,000	3	28,000	56	12,000	10	9,000	38
8,000	0	19,900	1	20,000	0	30,000	57	13,000	1	10,000	13

An examination of Table VI, in agreement with Table V, shows the germination to be greatly reduced by sodium chlorid in concentrations above 3,000 p. p. m., while it ceases entirely at about 6,000 p. p. m. With sodium carbonate a large reduction in germination occurred at about 10,000 p. p. m., but a few plants survived at about 20,000 p. p. m. The sodium sulphate showed about the same results as the sodium carbonate, while the magnesium sulphate gave over a 50 per cent germination at a concentration of 30,000 p. p. m. In the mixed salts the gypsum (calcium sulphate) did not have any great effect, possibly owing to the slowness with which gypsum dissolves.

On comparing the data in Tables V and VI with those reported in Table I and also others given later in the paper, where the salts were first dissolved and added in solution, it will be found that the salts were more toxic when added in solution than when mixed with the dry soil. This may be due to the slow solution and diffusion of the salt when added dry, which probably helps to explain the common observation that crops can sometimes be made to grow in a soil the analysis of which shows a very high total alkali content. It also explains why it is that crops growing on alkali land may look healthy and be growing vigorously until irrigated, when they are immediately killed.

In order to determine more exactly the effect of soil moisture on the toxicity of alkali salts, sand was placed in tin plates, as previously

described. To this sand salts were added in solution with the quantity of water necessary to bring the sand to the desired moisture content. Twenty-five kernels of Turkey Red wheat were planted in each pan, which was then covered with window glass to retain the moisture. Any loss in moisture was made up from time to time. The percentage of germination at the end of three weeks is given in Table VII.

TABLE VII.—Percentage of germination at the end of three weeks of the seeds of Turkey Red wheat in sand with different quantities of moisture and alkali salts. Salts added in solution

Salt and concentration (p. p. m. of dry soil).	Percentage of water in sand.				
	12	15	18	21	24
Sodium chlorid:					
None.....	75	80	84	84	78
800.....	92	80	72	88	80
1,800.....	48	80	88	76	48
2,400.....	28	60	88	80	60
2,900.....	4	24	68	64	44
3,600.....	0	0	84	12	16
4,000.....	0	0	36	0	8
4,500.....	0	0	6	0	0
5,700.....	0	0	4	0	0
6,000.....	0	0	0	0	0
Sodium carbonate:					
None.....	75	80	84	84	78
1,200.....	72	68	72	84	76
1,600.....	44	56	56	60	64
2,000.....	28	36	32	44	56
2,700.....	8	4	4	24	24
3,300.....	0	0	0	4	4
4,000.....	0	0	0	0	0
4,700.....	0	0	0	0	0
Sodium sulphate:					
None.....	75	80	84	84	78
2,000.....	88	88	92	96	88
4,000.....	36	72	80	92	68
6,000.....	12	60	72	72	72
8,000.....	8	4	20	44	64
10,000.....	0	0	28	36	36
12,000.....	0	0	0	12	20
14,000.....	0	0	0	0	4
16,000.....	0	0	0	0	4
18,000.....	0	0	0	0	0
Magnesium sulphate:					
None.....	75	80	84	84	78
12,000.....	20	24	40	28	56
14,000.....	16	12	48	48	60
16,000.....	12	16	48	52	48
18,000.....	4	8	20	44	40
20,000.....	0	4	8	16	48
22,000.....	0	0	0	12	12
24,000.....	0	0	0	12	12
26,000.....	0	0	0	0	4
28,000.....	0	0	0	0	0

From Table VII it will be seen that germination was first retarded by the salts when the soils contained but a small amount of moisture. With most of the salts the highest germination was in the wettest sand, while with sodium chlorid the intermediate moisture gave the highest germination.

It will be noted that in the sand sodium carbonate was more toxic than sodium chlorid. This same relation is also reported later in this paper with sand, although in all the tests with loam sodium chlorid was more toxic than sodium carbonate. A comparison of the limits of growth in sand with those already reported for loam brings out the fact that germination is reduced by a much lower concentration in sand than in loam. This is also brought out clearly in results reported later.

OUTLINE OF LATER WORK

GENERAL METHODS OF EXPERIMENTATION

A number of experiments were conducted in glass tumblers in which an equivalent of 200 gm. of dry soil was placed. Salts were added to the soil as follows: A stock solution of each salt was made up, containing an equivalent of 10 per cent of the anhydrous salt. The necessary quantity of the stock solution was then added to sufficient distilled water to make the soil up to 20 per cent water on the dry basis. The water containing the solution was thoroughly mixed with the soil on oilcloth and the whole placed in the glass. This method insured an even distribution of the salt through the soil.

In all cases the soil was made up to 20 per cent with moisture. This was about the optimum amount for plant growth. Ten seeds were planted in each glass to a depth of $\frac{1}{2}$ inch from the surface. After the seeds were planted the glass tumblers were covered with panes of window glass until the plants were up. This prevented evaporation and enabled the seeds to germinate with an even soil-moisture content.

Counts were made of the number of plants up each day, which made possible a determination of the relative time required for germination in the different treatments. The original moisture content was maintained by adding the necessary quantity of water every day or two. The plants were allowed to grow for two or three weeks, when they were harvested and measured and the dry weights determined.

The data obtained for each glass therefore included (1) the percentage of germination, (2) the average time required for germination, (3) the average height of plants, (4) the average number of leaves, and (5) the dry matter produced.

In each test there were 15 glasses for each concentration of salts, and there were 10 concentrations. In addition, there were four check glasses to which no salt was added. This made 154 glasses for each test. In the series there were 24 tests, which gave a total of 3,696 glasses.

Five determinations were made of the plants in each glass, making about 18,450 separate determinations. This number was reduced somewhat by the fact that plants did not germinate in all the glasses, owing to the high salt content. With this great number of results it is impracticable to give all the data in detail; hence, only summaries will be presented.

COMBINATION OF SALTS

In each test containing 15 glasses three different salts were used. The glasses were arranged in the triangular diagram used in expressing

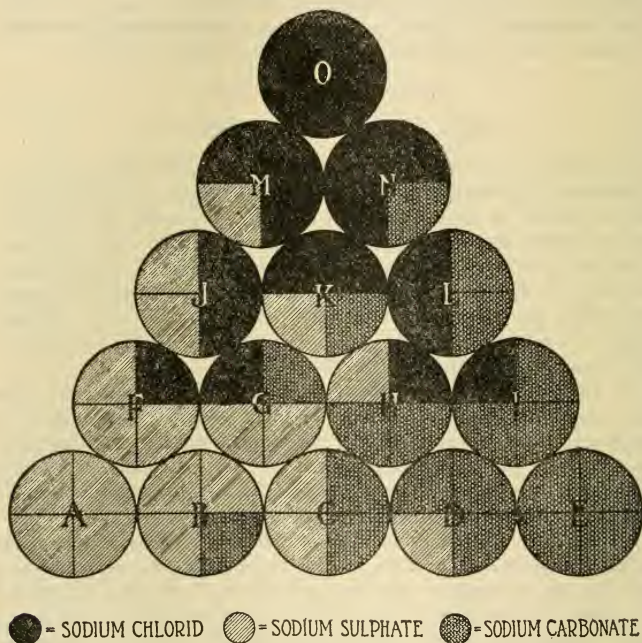


FIG. 1.—Diagram showing percentage of salts, mixtures, and their position in the diagrams of experimental sets. The arrangement of salts shown here is that in figure 2, page 14. The same positions but with different salts apply to figures 2 to 25.

three variables. This arrangement is shown in figure 1, the salts in this case being sodium sulphate, sodium carbonate, and sodium chlorid. All 15 glasses contain the same total concentration of salts—for example, in figure 1 the concentration is 1,000 parts of salt per million parts of dry soil.

The glasses on the corners of the diagram which are marked "A," "E," and "O" contained 100 per cent of the single salts. The other glasses

along the sides contained a mixture of two salts, while the glasses in the center contained all three salts in the proportions indicated.

It will be noted that the top glass (O) contained 100 per cent of sodium chlorid, the second row, with glasses M and N, 75 per cent of sodium chlorid, the third row, with glasses J, K, and L, 50 per cent of sodium chlorid, the fourth row, with glasses F, G, H, and I, 25 per cent of sodium chlorid, while the bottom row contained no sodium chlorid. The same order is followed with each of the other salts. Thus, there are glasses with each of the single salts, others with two salts in various combinations, and still others with all three salts in different proportions. From this arrangement it is possible to determine the effects of the single salts as well as the various combinations of salts.

In order to find the effects of the concentration of salts, 10 different concentrations were tried for each three salts. These varied from 1,000 to 10,000 p. p. m. of salt based on the dry soil. The combination of salts, as well as the soils and crops, are given in Table VIII.

TABLE VIII.—*Combinations of salts, soils, and crops used in concentration experiments*

Trial No.	Combination of salts.	Soil.	Crop.
1	Sodium chlorid, sodium sulphate, sodium carbonate..	Greenville loam.....	New Zealand wheat.
2	Potassium chlorid, calcium chlorid, magnesium chlorid.do.....	Do.
3	Sodium nitrate, potassium nitrate, magnesium nitrate.do.....	Do.
4	Sodium sulphate, potassium sulphate, magnesium sulphate.do.....	Do.
5	Potassium carbonate, sodium carbonate, ammonium carbonate (NH ₄) ₂ CO ₃ . ^ado.....	Do.
6	Sodium chlorid, sodium sulphate, sodium carbonate..	Coarse sand.....	Do.
7	Potassium chlorid, calcium chlorid, magnesium chlorid.do.....	Do.
8	Sodium nitrate, potassium nitrate, magnesium nitrate.do.....	Do.
9	Sodium sulphate, potassium sulphate, magnesium sulphate.do.....	Do.
10	Potassium carbonate, sodium carbonate, ammonium carbonate.do.....	Do.
11	Sodium chlorid, sodium sulphate, sodium carbonate..	College loam.....	Do.
12	Potassium chlorid, calcium chlorid, magnesium chlorid.do.....	Do.
13	Sodium nitrate, potassium nitrate, magnesium nitrate.do.....	Do.
14	Sodium sulphate, potassium sulphate, magnesium sulphate.do.....	Do.
15	Potassium carbonate, sodium carbonate, ammonium carbonate.do.....	Do.
16	Sodium chlorid, sodium sulphate, sodium carbonate..	Greenville loam.....	Chevalier barley.
17	Do.....do.....	White flint corn.
18	Do.....do.....	Danish oats.
19	Do.....do.....	Sugar beets.
20	Do.....do.....	Alfalfa.
21	Do.....do.....	Canada field peas.
22	Do.....	Distilled water.....	New Zealand wheat.
23	Potassium chlorid, calcium chlorid, magnesium chlorid.do.....	Do.
24	Sodium nitrate, potassium nitrate, magnesium nitrate.do.....	Do.

^a The ammonium carbonate used has the formula (NH₄)₂CO₃(NH₄)₂CO₂NH₂, but the simpler formula, (NH₄)₂CO₃, is used for convenience.

DESCRIPTION OF SOILS

The following analyses were made by members of the Utah Station staff from soils taken from the same fields as the soils used in the experiments. While the analyses are not of the exact soils used, they will be useful, since the soils in these fields are very uniform. See Tables IX and X.

TABLE IX.—*Chemical analysis of soils used (strong hydrochloric-acid digestion)*¹

Constituent.	Greenville soil.	College loam.	Sand.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Insoluble residue	42. 18	66. 69	51. 06
Potash (K ₂ O).....	. 67	. 55	. 15
Soda (Na ₂ O).....	. 35	. 49	. 21
Lime (CaO ₂).....	16. 88	7. 41	17. 43
Magnesia (MgO).....	6. 10	4. 15	5. 63
Iron oxid (Fe ₂ O ₃).....	3. 03	2. 93	. 86
Alumina (Al ₂ O ₃).....	5. 64	3. 49	1. 25
Phosphoric acid (P ₂ O ₅).....	. 41	. 25	. 14
Sulphuric acid (H ₂ SO ₄).....		. 07	. 03
Carbon dioxide (CO ₂).....	19. 83	7. 62	20. 73
Humus.....	. 53	2. 18	. 23
Total nitrogen.....	. 14	. 15	. 02

TABLE X.—*Physical analysis of soils used (determined with Yoder elutriator)*

¹ Constituent.	Greenville soil.	College loam.	Sand.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Coarse sand (above 1 mm.).....	9. 84	17. 69	70. 49
Fine sand (1 to 0.03 mm.).....	30. 04	37. 39	20. 75
Coarse silt (0.03 to 0.01 mm.).....	32. 25	15. 19	3. 32
Medium silt (0.01 to 0.003 mm.).....	12. 30	10. 36	1. 54
Fine silt (0.003 to 0. 001 mm.).....	6. 25	10. 32	. 81
Clay (below 0.001 mm.).....	7. 62	9. 03	2. 16
Real specific gravity.....	2. 67	2. 64	2. 81
Apparent specific gravity.....	1. 23	1. 32	1. 32

¹ For methods followed, see Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr., Bur. Chem., Bul. 107 (rev.), 272 p., 1908.

DETAILS OF GERMINATION OF PLANTS AND DRY MATTER PRODUCED

GREENVILLE SOIL

In accordance with the outline already given, five tests were made with Greenville soil, three different salts being used in each test. The arrangement of glasses, the number of seeds germinated, and the dry matter produced in each glass are given in figures 2, 3, 4, 5, and 6. The name of the salt is given at the corner of each triangle. The combination of

these salts can readily be determined by consulting figure 1. The number at the bottom of each triangle refers to the concentration of soluble salts in all the glasses of that triangle expressed in parts of anhydrous salt per million parts of dry soil.

An examination of figure 2 shows that some seeds germinated in all glasses up to a concentration of 4,000 p. p. m., but that at 5,000 p. p. m. there was no germination in the glass having all sodium chlorid, and only germination in one of the glasses with three-fourths sodium chlorid. In the part of the triangle toward the sodium chlorid the germination gradually decreased as the concentration increased. The sodium carbonate and sodium sulphate showed almost a complete germination up to 10,000 p. p. m., or 1 per cent of salt.

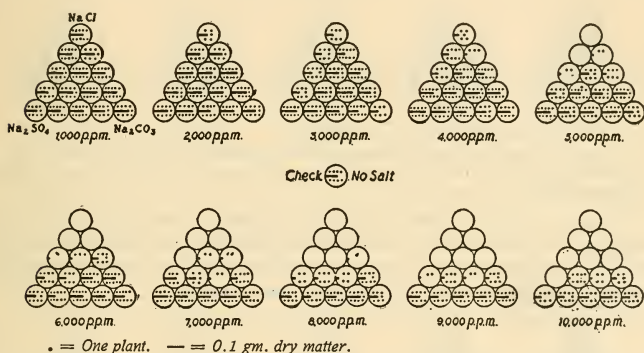


FIG. 2.—Diagram showing the number of wheat plants up and dry matter produced in 24 days on Greenville loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

The greater toxicity of sodium chlorid as compared with sodium carbonate was somewhat of a surprise, since most of the literature on alkali considers sodium carbonate, or black alkali, as being by far the most harmful of the alkali salts. The results given here agree with those found in the experiments of 1912 and 1913 and are also borne out by the results shown in figures 7, 12, 17, 18, 19, 20, 21, and 22, where different crops are compared.

In the glasses that received sodium carbonate the surface was black with dissolved humus and was somewhat crusted, showing that the physical condition had been injured. Notwithstanding this fact, seeds germinated in the soil and the plants grew for three weeks with no great injury except a slight blackening of plants at the surface of the soil with higher concentrations.

Figure 3 shows results for the chlorids of potassium, calcium, and magnesium. These chlorids are not as toxic as the chlorid of sodium,

but they are all more toxic than the sodium sulphate and sodium carbonate. Magnesium chlorid seemed to be the least toxic of the chlorids that were tested. Germination in all of them fell off rapidly above 4,000 p. p. m.

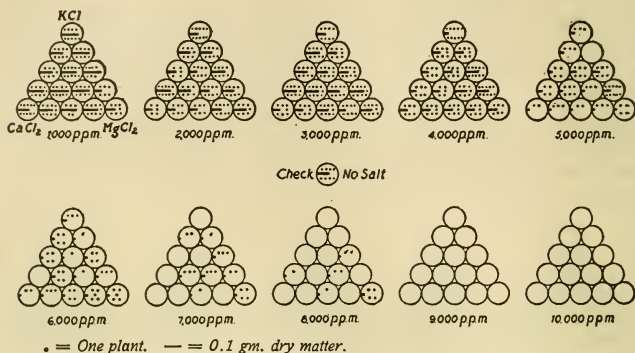


FIG. 3.—Diagram showing the number of wheat plants up and dry matter produced in 24 days on Greenville loam with calcium chlorid, magnesium chlorid, and potassium chlorid in different combinations and concentrations.

In figure 4 the nitrates of sodium, potassium, and magnesium are compared and the sodium found to be slightly more toxic than the others. The nitrates appear on the whole to be somewhat less toxic than the chlorids, but more so than the sulphates or carbonates.

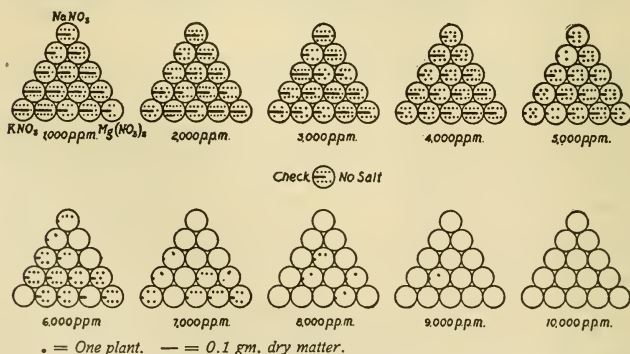


FIG. 4.—Diagram showing the number of wheat plants up and dry matter produced in 24 days on Greenville loam with potassium nitrate, magnesium nitrate, and sodium nitrate in different combinations and concentrations.

The results for the sulphates of sodium, potassium, and magnesium are given in figure 5. There was practically complete germination with all of the sulphates up to a concentration of 1 per cent; hence, but little difference in the three salts can be seen.

With the carbonates shown in figure 6 there is a marked falling off with the ammonium carbonate above 5,000 p. p. m. With the others there is a good germination up to 10,000 p. p. m., similar to the results shown in figure 2. The formula given by the manufacturers of the ammonium

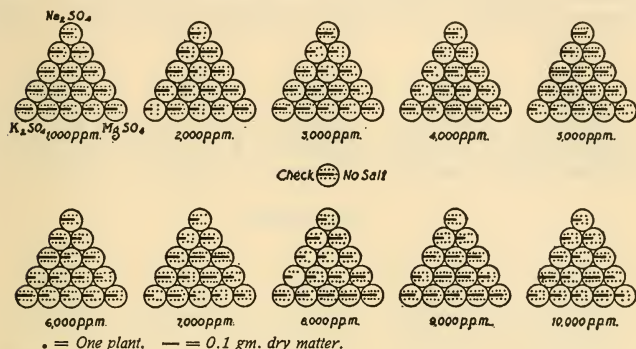


FIG. 5.—Diagram showing the number of wheat plants up and dry matter produced in 24 days on Greenville loam with potassium sulphate, magnesium sulphate, and sodium sulphate in different combinations and concentrations.

carbonate was (NH₄)₂CO₃(NH₄)CO₂NH₂ instead of the shorter formula, (NH₄)₂CO₃, given on the figures.

It is probable that the toxicity of the ammonium carbonate was due, in part at least, to the free ammonia that was constantly being given off

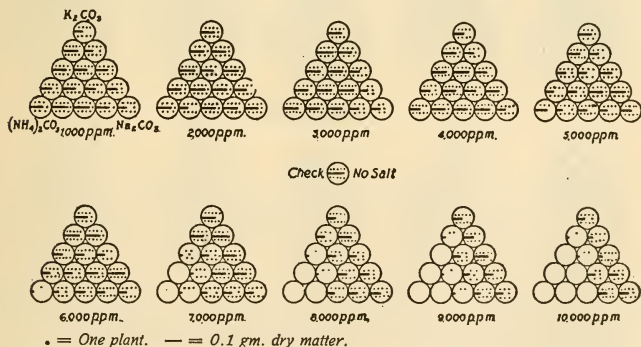


FIG. 6.—Diagram showing the number of wheat plants up and dry matter produced in 24 days on Greenville loam with ammonium carbonate, sodium carbonate, and potassium carbonate in different combinations and concentrations.

by this unstable compound rather than to the CO₂ part of the compound. It is a well-known fact that protoplasm is very sensitive to the action of free ammonia.

SAND

Five sets of tests were conducted with wheat growing in sand similar to those with the Greenville soil.

In figure 7 the results for sodium chlorid, sodium sulphate, and sodium carbonate are given. The noticeable thing about these results, as well

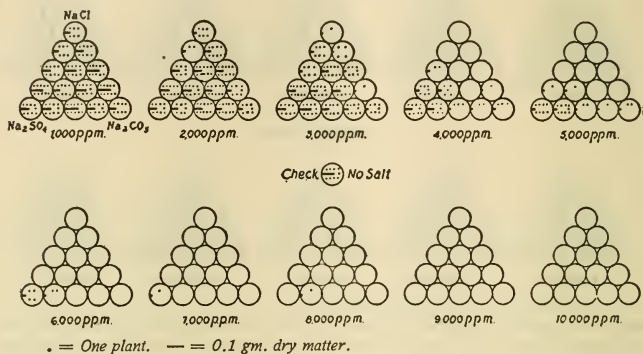


FIG. 7.—Diagram showing the number of wheat plants up and dry matter produced in 14 days on coarse sand with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

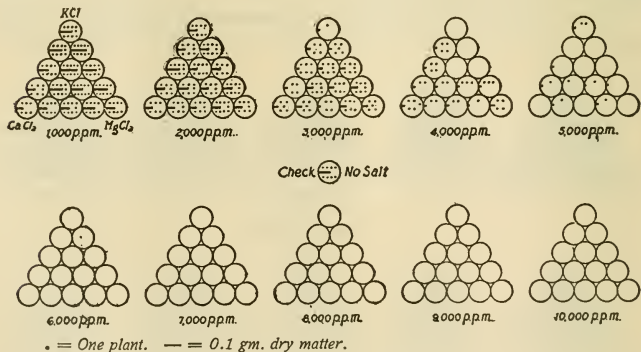


FIG. 8.—Diagram showing the number of wheat plants up and dry matter produced in 14 days on coarse sand with calcium chlorid, magnesium chlorid, and potassium chlorid in different combinations and concentrations.

as all those for sand, is that only about half as much salt is required to stop growth in sand as in either the Greenville soil or the College loam.

The same general relations between the salts are shown here as in the Greenville soil, except that in the sand sodium carbonate is propor-

tionately more toxic than in the other soils. This is exactly the same result that was obtained in 1913 in the experiments already described. In sand the carbonates seem to be nearly as toxic as the chlorids, while in the other soil they are very much less injurious.

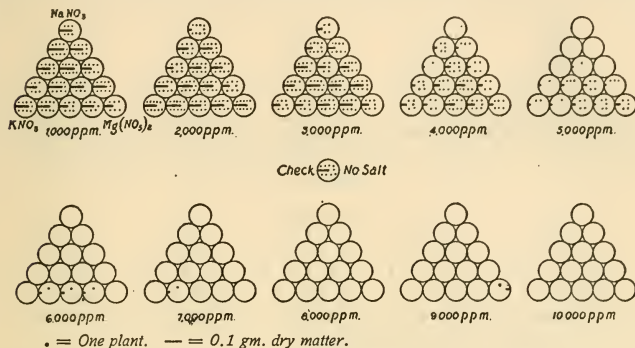


FIG. 9.—Diagram showing the number of wheat plants up and dry matter produced in 14 days on coarse sand with potassium nitrate, magnesium nitrate, and sodium nitrate in different combinations and concentrations.

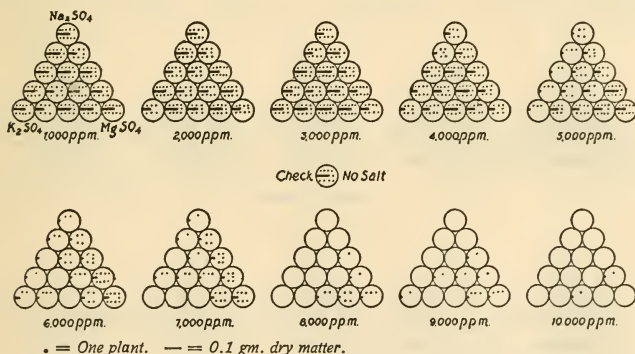


FIG. 10.—Diagram showing the number of wheat plants up and dry matter produced in 14 days on coarse sand with potassium sulphate, magnesium sulphate, and sodium sulphate in different combinations and concentrations.

Figure 8 shows the same relationship between the chlorids as was brought out in figure 3. It also shows that these salts are in injurious lower concentrations in sand than in other soils.

The nitrates are shown in figure 9 to be slightly less injurious than the chlorids in figure 8. The sodium salt is again shown to be more injurious than the others.

In sand the limit of growth in the presence of sulphates is shown by figure 10 to be less than 10,000 p. p. m., while in the loam growth was scarcely retarded at this concentration. Plants seem able to resist decidedly more magnesium sulphate than either potassium sulphate or

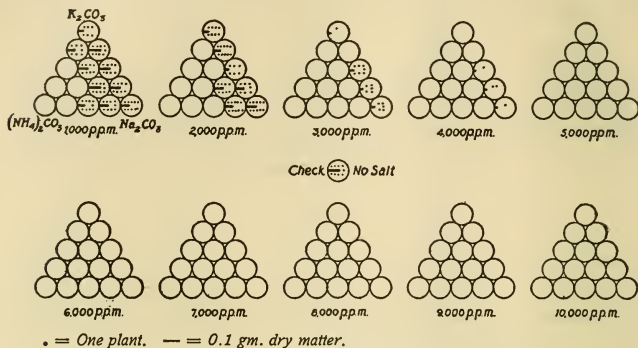


FIG. 11.—Diagram showing the number of wheat plants up and dry matter produced in 14 days on coarse sand with ammonium carbonate, sodium carbonate, and potassium carbonate in different combinations and concentrations.

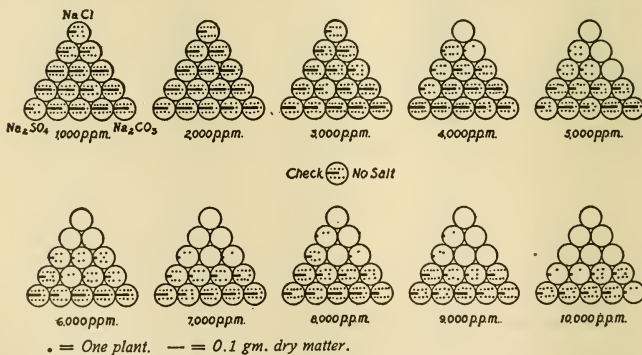


FIG. 12.—Diagram showing the number of wheat plants up and dry matter produced in 16 days on College loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

sodium sulphate. This is in accord with the earlier results found in 1912 and 1913.

Figure 11 shows that there was no germination whatever in sand where even as little as 1,000 p. p. m. of ammonium sulphate were found. With

any of the carbonates there was no germination for concentrations above 4,000 p. p. m.

COLLEGE LOAM

The same number of tests, using the same kinds of salts and seeds were conducted in College loam as in Greenville soil and sand. The

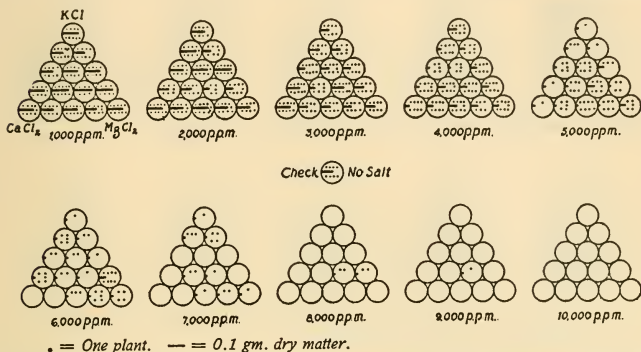


FIG. 13.—Diagram showing the number of wheat plants up and dry matter produced in 16 days on College loam with calcium chlorid, magnesium chlorid, and potassium chlorid in different combinations and concentrations.

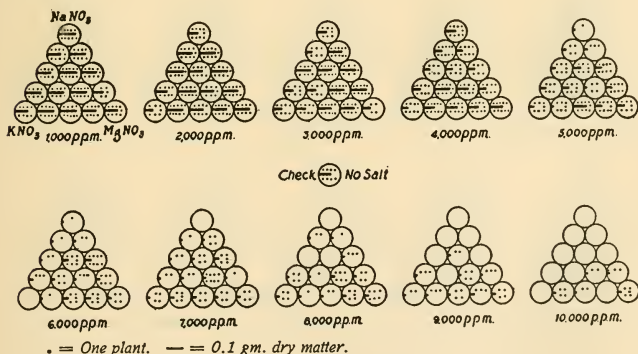


FIG. 14.—Diagram showing the number of wheat plants up and dry matter produced in 16 days on College loam with potassium nitrate, magnesium nitrate, and sodium nitrate in different combinations and concentrations.

results are shown in figures 12, 13, 14, 15, and 16. These results agree so completely with those found for the Greenville soil that individual comment seems unnecessary.

COMPARISON OF CROPS

In the management of alkali land it is important to know the relative resistances of various crops. Farmers who have been accustomed to deal with alkali are well aware that certain crops can be made to grow where others would be a complete failure.

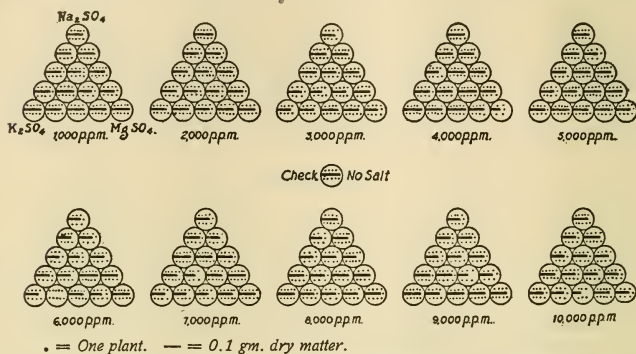


FIG. 15.—Diagram showing the number of wheat plants up and dry matter produced in 16 days on College loam with potassium sulphate, magnesium sulphate, and sodium sulphate in different combinations and concentrations.

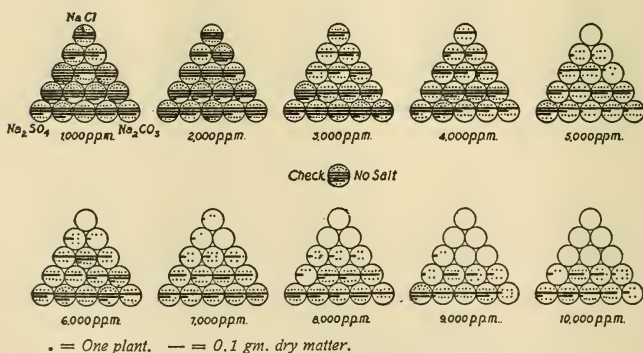


FIG. 16.—Diagram showing the number of wheat plants up and dry matter produced in 16 days on College loam with ammonium carbonate, sodium carbonate, and potassium carbonate in different combinations and concentrations.

A number of the common field crops were tested in the manner already described. Greenville soil was placed in glass tumblers and sodium chlorid, sodium sulphate, and sodium carbonate added in the same combinations and concentrations previously used. Ten seeds were

planted in each glass. The crops compared were wheat (*Triticum* spp.), barley (*Hordeum* spp.), oats (*Avena sativa*), corn (*Zea mays*), alfalfa (*Medicago sativa*), sugar beets (*Beta vulgaris*), and Canada field peas (*Pisum arvense*). The results for wheat have already been shown in

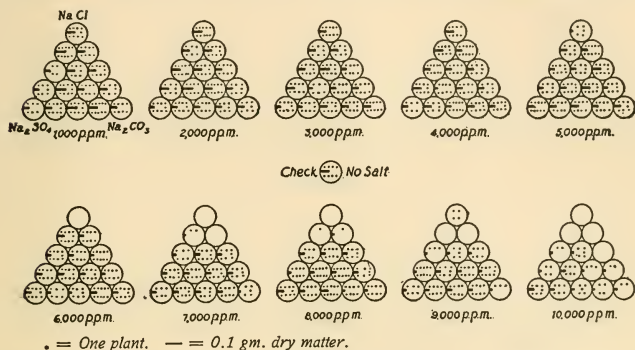


FIG. 17.—Diagram showing the number of barley plants up and dry matter produced in 24 days on Greenville loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

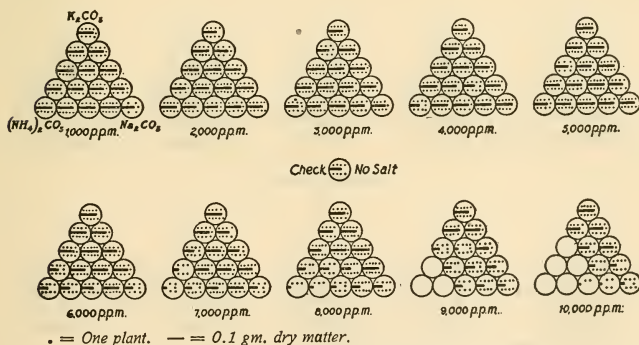


FIG. 18.—Diagram showing the number of corn plants up and dry matter produced in 21 days on Greenville loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

figure 2, while those for the other crops will be found in figures 17, 18, 19, 20, 21, and 22.

An examination of these diagrams shows that the relation between the salts, pointed out in connection with wheat, holds for the other crops.

According to the resistance of their seedlings to alkali, the crops fall into the following order: (1) Barley, (2) oats, (3) corn, (4) wheat, (5)

sugar beets, (6) alfalfa, and (7) Canada field peas. It may be that after the crops get a good start their resistance would not be in just this order; but in the percentage of seeds germinated this order seems to hold. Barley was able to withstand about twice as much alkali as field peas.

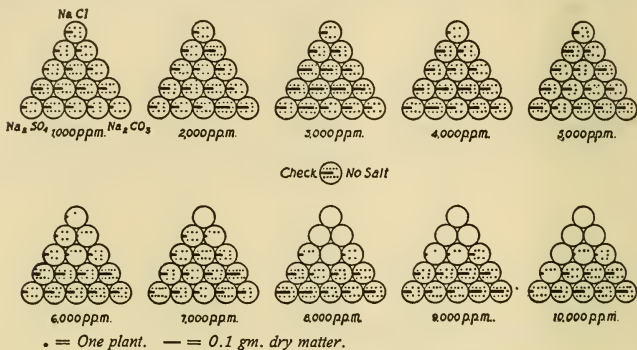


FIG. 19.—Diagram showing the number of oat plants up and dry matter produced in 21 days on Greenville loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

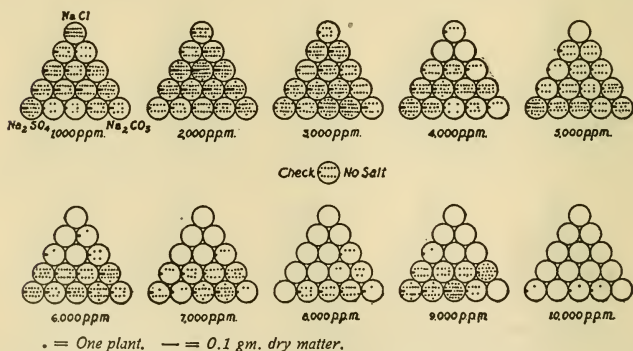


FIG. 20.—Diagram showing the number of sugar-beet plants up and dry matter produced in 21 days on Greenville loam with sodium sulphate, sodium carbonate, and sodium chlorid in different combinations and concentrations.

SOLUTION CULTURES

In order to compare the effect of salts in solution cultures with the same salts in soils, a number of tests were made with seedlings growing in distilled water to which various salts had been added. Glass tumblers were filled with water containing the proper quantity of the desired

solution. The glasses were then covered with paraffined paper which was bent over the edges and held in place by rubber bands. New Zealand wheat was germinated between moist filter papers until its roots were about half an inch long, when 10 seedlings to each glass were placed in

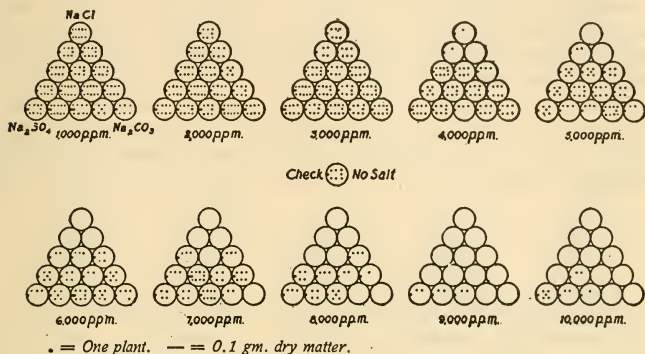


FIG. 21.—Diagram showing the number of alfalfa plants up and dry matter produced in 21 days on College loam with sodium sulphate, sodium carbonate, and sodium chloride in different combinations and concentrations.

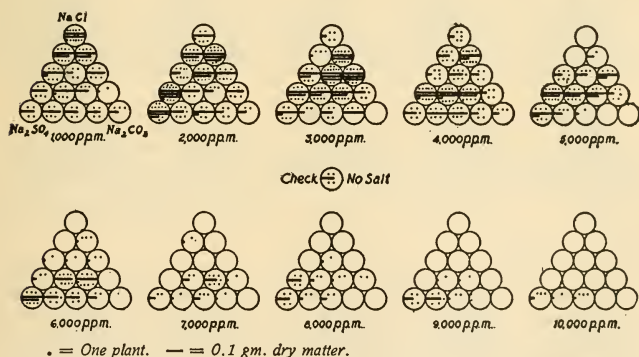


FIG. 22.—Diagram showing the number of Canada field-pea plants up and dry matter produced in 21 days on Greenville loam with sodium chloride, sodium sulphate, and sodium carbonate in different combinations and concentrations.

holes in the paraffined paper, so that their roots grew down into the solutions.

The loss of water due to transpiration was made up every day or two.

The glasses were arranged in the triangular diagram as in the experiments with soils, which have already been discussed. In each test the

concentrations ranged from 1,000 parts of anhydrous salt for each 1,000,000 parts of water up to 10,000 p. p. m. of salt.. The seedlings were allowed to grow 21 days before being harvested. At harvest the following determinations were made of the plants in each glass: (1) Plants still alive, (2) average height of plants, (3) average length of roots, (4) average number of leaves per plant, (5) dry weight of tops, (6) dry weight of roots, (7) ratio of length of tops to length of roots, (8) ratio of weight of tops to weight of roots.

In the first test sodium chlorid, sodium carbonate, and sodium sulphate, were used; in the second, potassium chlorid, calcium chlorid, and magnesium chlorid; and in the third, sodium nitrate, potassium nitrate, and magnesium nitrate. Figures 23, 24, and 25 show in detail the number of

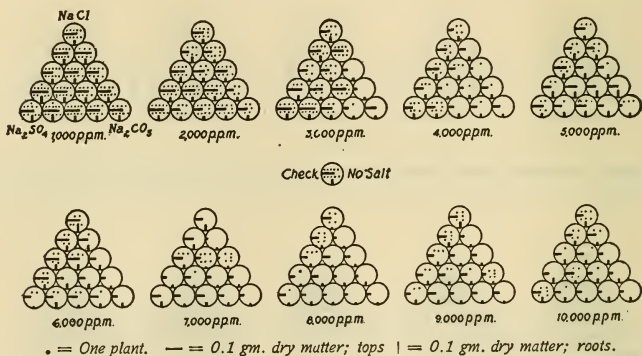


FIG. 23.—Diagram showing the number of seedlings alive and dry matter produced in tops and roots in 21 days with solutions of sodium chlorid, sodium sulphate, and sodium carbonate in different combinations and concentrations.

plants alive at the end of three weeks, as well as the weight of tops and roots in each glass.

An examination of the figures shows a gradual decrease in growth as the concentration of salts increased. Plants were able to endure much stronger chlorids and nitrates in solution culture than in the soil, while the carbonate retarded growth more in the solution than in the loam, but not as much as in the sand. The plants growing in the distilled water without any salts had no food except that stored in the seed and that dissolved from the glass, and, as a result, they produced less growth than plants growing in the dilute solutions.

The results showing the effect of concentration of the various salts are summarized in Table XI. Each figure represents the average of nine different salts of a given concentration. An examination of the table shows that the number of plants alive at the end of three weeks

decreased as the concentration of the solution increased, there being an average of 9.7 plants to each glass alive where no salt was added to the culture, but only 3.8 plants alive with 10,000 p. p. m. of salt.

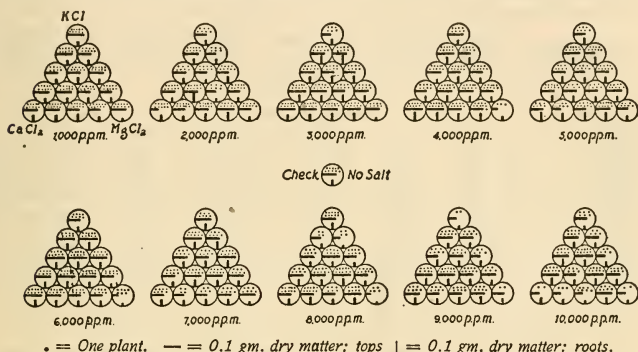


FIG. 24.—Diagram showing the number of wheat seedlings alive and dry matter produced in tops and roots in 21 days with solutions of potassium chlorid, calcium chlorid, and magnesium chlorid in different combinations and concentrations.

There was a corresponding decrease in number of leaves per plant, height of plants, length of roots, weight of tops, and weight of roots as the concentration of salts increased. The weight of roots, however, was not so much affected as some of the other results. In the cultures in

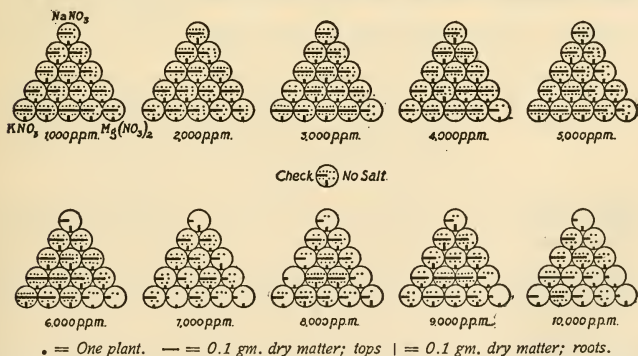


FIG. 25.—Diagram showing the number of wheat seedlings alive and dry matter produced in tops and roots in 21 days with solutions of sodium nitrate, potassium nitrate, and magnesium nitrate in different combinations and concentrations.

which no salts were added, the height of plants, the length of roots, and the dry matter produced were not so great as in the cultures containing salts in low concentrations.

TABLE XI.—Effect of concentration of salts in solution cultures on the growth of wheat seedlings. Average of 45 glasses for each concentration, with sodium sulphate, sodium carbonate, sodium chlorid, calcium chlorid, potassium chlorid, potassium nitrate, magnesium nitrate, and sodium nitrate in various combinations

Concentration of salts in solution.	Number of plants alive.	Number of leaves per plant.	Height of plants.	Length of roots.	Ratio of height to length of root.	Dry weight of tops.	Dry weight of roots.	Ratio of weight of tops to roots.
<i>P. p. m.</i>			<i>Inches.</i>	<i>Inches.</i>		<i>Gm.</i>	<i>Gm.</i>	
None.....	9.7	1.97	7.5	3.9	1.92:1	0.123	0.052	2.36:1
1,000.....	9.0	1.91	8.6	4.4	1.91:1	.143	.046	3.37:1
2,000.....	7.8	1.72	6.8	3.4	1.96:1	.123	.044	3.04:1
3,000.....	5.1	1.67	6.8	3.6	1.88:1	.137	.048	3.08:1
4,000.....	5.7	1.41	6.0	3.2	1.87:1	.123	.045	2.83:1
5,000.....	5.7	1.70	5.4	3.0	1.88:1	.118	.052	2.40:1
6,000.....	5.8	1.62	5.7	3.1	1.90:1	.133	.050	2.67:1
7,000.....	4.1	1.34	4.6	2.8	1.81:1	.100	.040	2.43:1
8,000.....	4.3	1.43	4.1	2.3	1.74:1	.096	.038	2.46:1
9,000.....	4.4	1.37	4.1	2.3	1.74:1	.105	.040	2.58:1
10,000.....	3.8	1.29	3.2	2.0	1.70:1	.100	.043	2.37:1

Table XII shows the effect of the individual salts when used alone. The results given in this table are the averages of various concentrations, from 1,000 to 10,000 p. p. m. In interpreting these figures it must be remembered that no nutrient solution was added where the single salt was present. Using the average height of plants as an index, the toxicity of the salts was in the following order: Sodium carbonate, sodium chlorid, magnesium nitrate, sodium sulphate, magnesium chlorid, sodium nitrate, potassium nitrate, potassium chlorid, and calcium chlorid.

TABLE XII.—Growth of wheat seedlings in solution cultures of various salts. Average of 10 concentrations of each salt

Salt.	Number of plants alive.	Average leaves per plant.	Height of plants.	Length of roots.	Ratio of height to root length.	Dry weight of tops.	Dry weight of roots.	Ratio of weight of tops to roots.
			<i>In.</i>	<i>In.</i>		<i>Gm.</i>	<i>Gm.</i>	
Sodium sulphate.....	4.8	1.4	4.2	2.2	1.91:1	0.096	0.044	2.18:1
Sodium carbonate.....	1.7	1.2	2.1	1.6	1.31:1	.063	.028	2.25:1
Sodium chlorid.....	5.2	1.3	3.1	2.0	1.55:1	.092	.046	2.00:1
Calcium chlorid.....	8.4	1.8	7.9	3.2	1.88:1	.130	.066	1.97:1
Magnesium chlorid.....	6.0	1.6	5.0	1.5	3.33:1	.109	.036	3.03:1
Potassium chlorid.....	7.1	1.6	6.2	2.6	2.38:1	.126	.051	2.47:1
Potassium nitrate.....	6.0	1.8	5.8	2.7	2.15:1	.154	.039	3.95:1
Magnesium nitrate.....	2.5	1.3	3.4	1.5	2.27:1	.073	.031	2.35:1
Sodium nitrate.....	4.4	1.5	5.4	2.7	2.00:1	.113	.041	2.76:1

A rather conspicuous point in the table is the high ratio of tops to roots, both as to length and weight, in the cultures containing magnesium chlorid. The roots were also very short with magnesium nitrate,

even more so than with sodium carbonate. This affirms the well-known toxicity of magnesium salts to roots when used alone. The various salts in solution cultures did not act at all in the same manner as in soils, which shows the inadvisability of applying too widely to the soil the results obtained with solution cultures of alkali.

RESULTS OF STUDIES

NUMBER OF SEEDS GERMINATED

In the five graphs which follow (fig. 26-30) the effects of various factors on the number of seeds germinating in each glass are given. These are all summaries and each one represents a great many figures. It will be remembered that 10 seeds were planted in each glass.

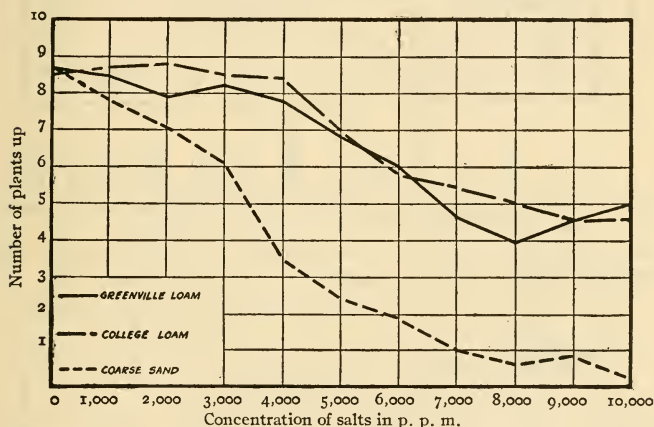


Fig. 26.—Curve showing the number of wheat plants germinating in College loam, Greenville loam, and sand with different concentrations. Average of 13 salts.

Figure 26 shows the effect of the concentration of salts in sand, Greenville loam, and College loam on the number of seeds germinating. Each curve represents the average of 13 salts in various combinations. In all of the soils there was an average of about $8\frac{1}{2}$ plants coming up in each glass to which no salt was added. In sand the germination rapidly decreased with the concentration of salt, especially above 3,000 p. p. m. In College loam and Greenville loam there was but little falling off in germination until a concentration of over 4,000 p. p. m. had been reached.

Figure 27 shows the effect of the various salts on the germination of wheat in the three kinds of soil. Each salt represents the average of 10 concentrations ranging from 1,000 to 10,000 p. p. m. In sand there

was no germination whatever when ammonium carbonate was present even in as low a concentration as 1,000 p. p. m., but in the loams this

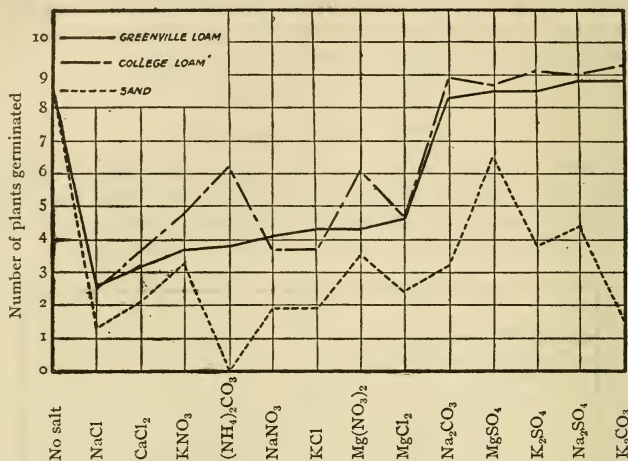


FIG. 27.—Curve showing the number of wheat plants germinating in College loam, Greenville loam, and sand containing various salts. Average for all concentrations.

salt was not so toxic as some of the chlorids. The salts are arranged in the order of their toxicity to germination in Greenville loam.

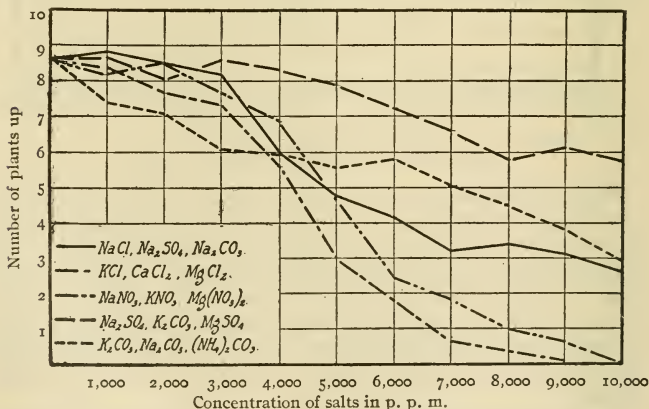


FIG. 28.—Curve showing the effect of various combinations of salts in different concentrations on the number of wheat plants germinating. Average of 15 combinations.

Figure 28 gives results where three salts were present in the soils in various combinations. Potassium chlorid, calcium chlorid, and mag-

nesium chlorid retarded germination most of any of the salts that were used together, while sodium sulphate, potassium sulphate, and magnesium sulphate retarded it least. With the first three salts there was no germination whatever above 9,000 p. p. m. and less than one-third complete germination at a concentration of 5,000 p. p. m.

In figure 29 the effect of the concentration of sodium chlorid, sodium carbonate, and sodium sulphate on the different crops is shown. A striking feature of the table is the stimulating effect of these salts in low concentration on the germination of sugar beets. With the exception of sugar beets, all the crops showed considerable similarity. One reason for the high germination of beets is the number of germs in each seed

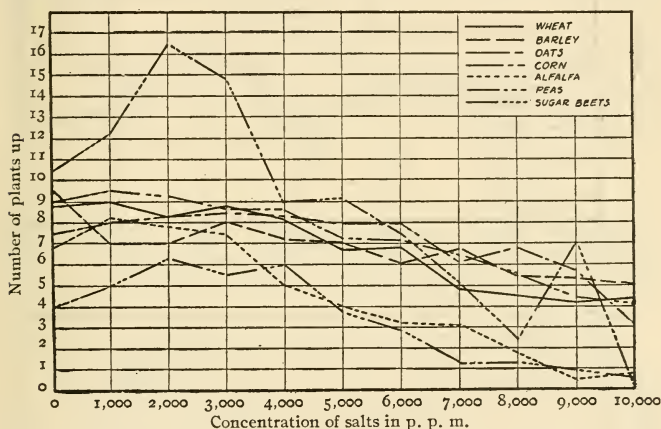


FIG. 29.—Curve showing the effect of concentration of salts on the number of seeds of various kinds germinating. Average for sodium chlorid, sodium carbonate, and sodium sulphate.

ball. Alfalfa and field peas were affected by the salts decidedly more than the cereals.

The individual effect of sodium chlorid, sodium carbonate, and sodium sulphate on the different crops is shown in figure 30. Sodium chlorid is seen to be rather uniformly toxic to all crops, while sodium carbonate varies greatly. Sugar beets seem to be particularly resistant to sodium sulphate.

DRY MATTER PRODUCED

The five curves which follow (fig. 31-35) show the same results for amounts of dry matter produced by each glass that were given for germination in the five preceding figures (fig. 25-30). The numbers given represent the dry weight of plant material produced in each glass.

Figure 31 shows that the production of dry matter was stimulated by the presence of 1,000 p. p. m. of salt in the Greenville and College loam, but was about the same in sand for 1,000 p. p. m. as where no salt was

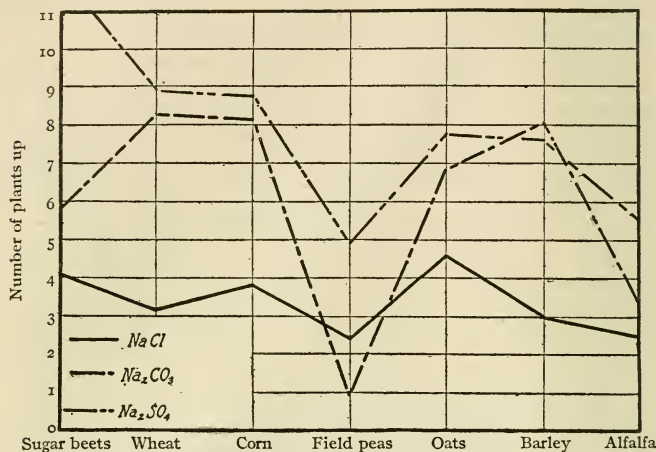


FIG. 30.—Curve showing the effect of sodium chloride, sodium carbonate, and sodium sulphate on the number of plants up from seeds of various kinds. Average for concentrations from 1,000 to 10,000 p. p. m.

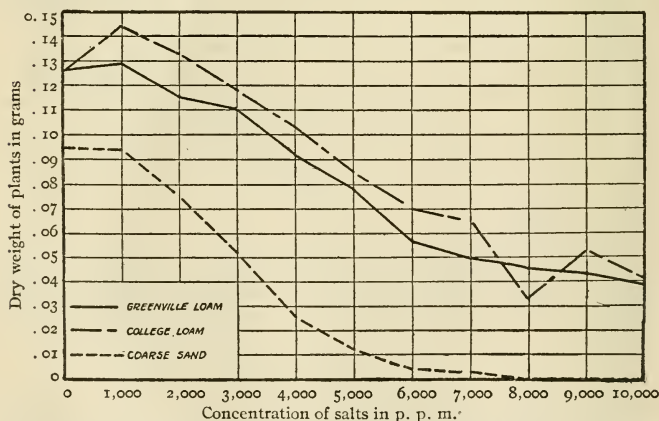


FIG. 31.—Curve showing the dry weight of wheat plants germinating in College loam, Greenville loam, and sand with different concentrations. Average of 13 salts.

added. The quantity of dry matter rapidly decreased with the concentration of salt above this point. In sand there was no plant growth at all above 8,000 p. p. m. of salt.

The effect of individual salts is shown in figure 32. A comparison of this graph with figure 27 shows that the dry matter is affected by the

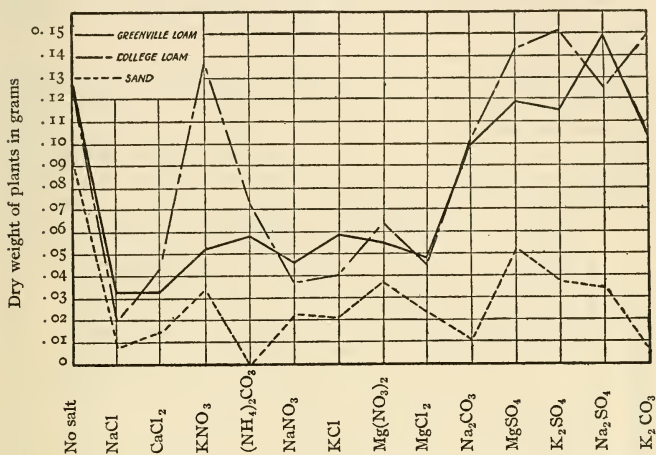


FIG. 32.—Curve showing the dry weight of wheat plants germinating in College loam, Greenville loam, and sand containing various salts. Average for all concentrations.

salt in just about the same way as the germination. The greater relative toxicity of the carbonates in sand than in loam is again brought out.

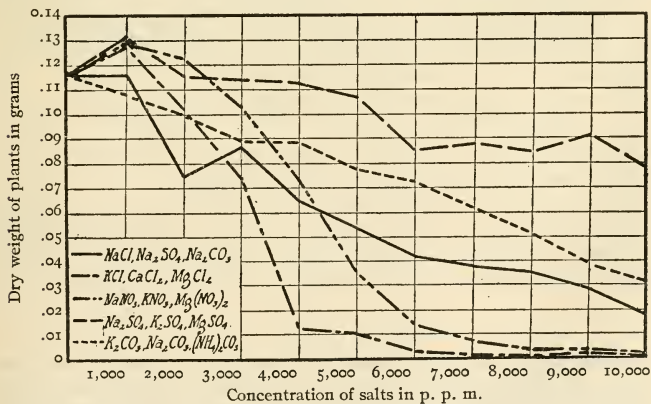


FIG. 33.—Curve showing the effect of various combinations of salts in different concentrations on the amount of dry weight produced. Average of 15 combinations of each 3 salts.

The action of each three salts used together is shown in figure 33. With the exception of potassium carbonate, sodium carbonate, and am-

monium carbonate the production of dry matter was stimulated by low concentrations of the salts. The growth of plants was not greatly reduced by the sulphates even in relatively high concentrations, while with the chlorids the yield dropped very rapidly and was practically nothing where the concentration was above 4,000 p. p. m.

Figure 34 shows the dry matter produced by different kinds of crops in soils containing sodium chlorid, sodium carbonate, and sodium sulphate in concentrations from 1,000 to 10,000 p. p. m. Corn gave by far the largest quantity of dry matter, but it was probably as much affected by the salt as any other crop. The yield was reduced from above 0.6 gm. per glass with no salt to less than 0.1 gm. per glass with a concentration of 10,000 p. p. m. Canada field peas produced a large quantity of dry

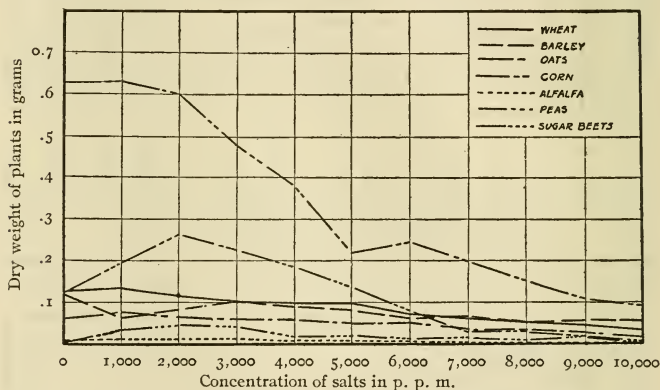


FIG. 34.—Curve showing the effect of concentration of salts on the dry weight of plants from seeds of various kinds. Average for sodium chlorid, sodium carbonate, and sodium sulphate.

matter, but they were also greatly affected by the concentration of salt. Alfalfa gave the least total yield under all conditions.

The effect of the individual salts on the yield of the various crops is brought out in figure 35. The yield of all crops was highest with sodium sulphate and lowest with sodium chlorid. With most crops it was only about half as great with sodium chlorid as with sodium carbonate.

DAYS TO COME UP

During the experiments a count was made each day of the number of plants that appeared above the surface of the soil, and from these figures a determination was made of the average time required for the plants in each glass to come up. The average results are in some cases misleading, because with toxic salts no plants germinated in the high concentration, and the averages were determined from the plants that came

up, which in this case were only those in low concentrations. At the same time there might be considerable germination in the high concentrations of less toxic salts, but the time of germination was increased. Thus, the average time of germination might appear to be longer in the less toxic salt, when in reality this would not be the case.

Figure 36 shows the time required for wheat to come up in Greenville loam, College loam, and sand containing salts in concentrations up to 10,000 p. p. m. The results are the average for 13 different salts. The time required to germinate where no salt was present varied from about $5\frac{1}{2}$ to $6\frac{1}{2}$ days with no salt and from $10\frac{1}{2}$ to 15 days with 10,000 p. p. m.

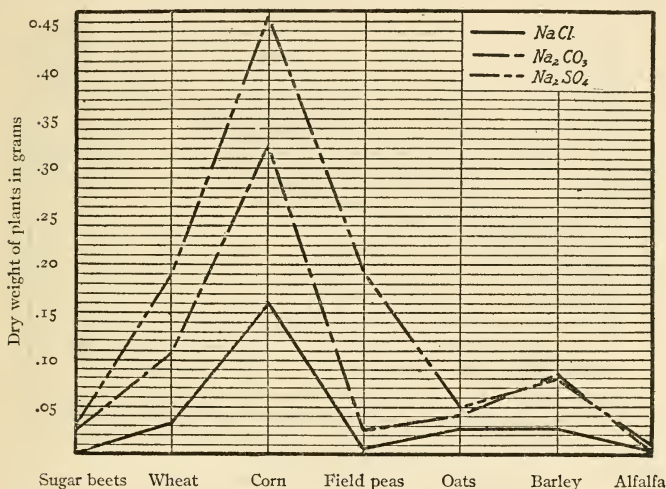


FIG. 35.—Curve showing the effect of sodium chlorid, sodium carbonate, and sodium sulphate on the dry weight from seeds of various kinds. Average for concentrations from 1,000 to 10,000 p. p. m.

of salt. The time was doubled by the presence of from 6,000 to 8,000 p. p. m. of salt.

Figure 37 shows the effect of individual salts on time of germination in the three kinds of soil. Calcium chlorid, magnesium chlorid, and sodium chlorid retarded germination most in Greenville soil, while sodium nitrate came next.

In sand the salts did not retard germination as much as in loam. This is because there was no germination whatever in sand with the highest concentration. There was no germination in sand when ammonium carbonate was added, even in as low concentrations as 1,000 p. p. m.

The results where three salts were used together are shown in figure 38. The average time of germination with potassium chlorid, calcium

chlorid, and magnesium chlorid in a concentration of 8,000 p. p. m. was over 20 days, which was nearly four times as long as the time required for seeds to come up where no salt was added. The period of germination was less with the sulphates and carbonates than with the other salts.

The time of germination of different crops in the presence of sodium chlorid, sodium carbonate, and sodium sulphate in combination is shown in figure 39. Where no salts were added, the time varied from about $4\frac{1}{2}$ days for barley to nearly 8 days for sugar beets. The same general relation between the germination of various crops continued with the different concentrations of salts. Alfalfa was least affected by salts of any of the crops in the length of its germination period.

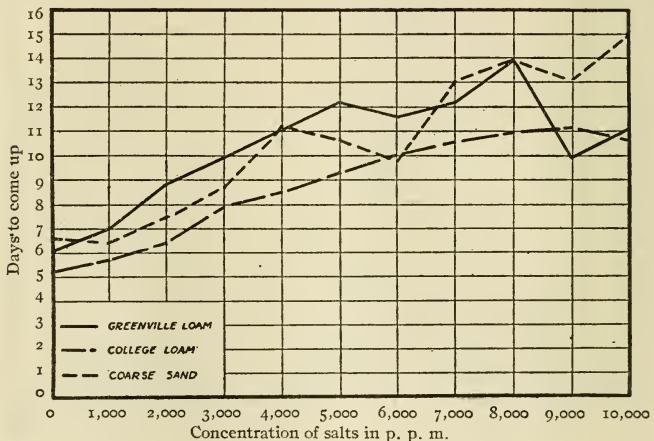


FIG. 36.—Curve showing the number of days for wheat plants to come up in College loam, Greenville loam, and sand with different concentrations. Average of 13 salts.

Figure 40 shows the effects of individual salts on the germination period of different crops. This brings out again the fact already mentioned, that the same relative toxicity of salts does not hold for all crops.

HEIGHT OF PLANT

Figures 41, 42, 43, 44, and 45 show the effect of various factors on the height of plants. This is probably one of the best means of comparison for young plants of this kind.

Plants growing in sand were not so high in any case as those growing in other soils; in the Greenville loam they were slightly higher than in College loam. The height in loam was greater with 1,000 p. p. m. of salt than where no salt was added, but above this point the height decreased considerably as the concentration of salt increased. In sand

the height was much more affected by the salts than in loam. The rise in the curve at 10,000 p. p. m. is due to the fact that no plants grew at this concentration in the more toxic salts and not to the actual increase in height.

Figure 42 shows the effect of each salt in the three soils on the height of wheat. The same general results which have already been pointed out in connection with germination and dry-matter production are noted here. Potassium nitrate produced the shortest plants in the loams,



FIG. 37.—Curve showing the number of days for wheat plants to come up in College loam, Greenville loam, and sand containing various salts. Average for all concentrations.

while sodium chlorid and sodium carbonate produced the shortest plants in sand.

Figure 43 shows the height of plants in soils to which three salts in combinations of various kinds had been added. This diagram shows that the chlorids and nitrates had a great effect on the height of plants, while the carbonates and sulphates had less.

The effect of the concentrations of sodium chlorid and sodium sulphate on the height of different crops is shown in figure 44. While the curves are somewhat irregular, they show the same results that have already been brought out regarding the shortening of plants by alkali.

Figure 45 shows the effect of individual salts on the height of various crops. It will be noted that in practically all cases the crops were shorter where sodium chlorid was present than with the other salts; also that sodium sulphate usually gave the highest plants.

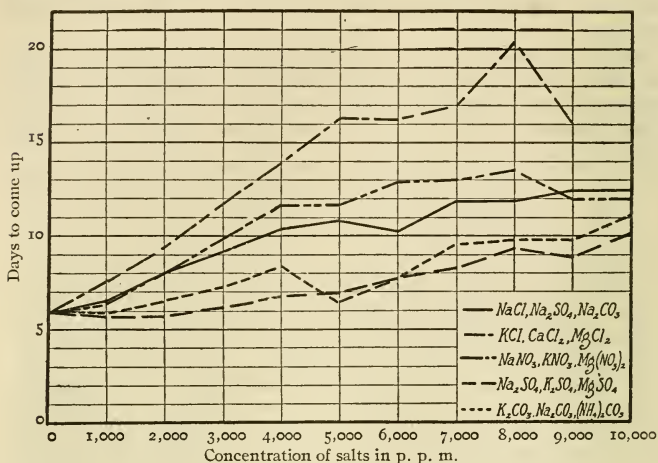


FIG. 38.—Curve showing the effect of various combinations of salts in different concentrations on the number of days to come up. Average of 15 combinations.

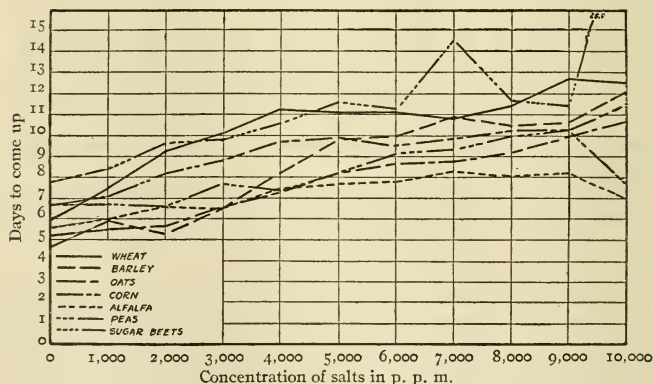


FIG. 39.—Curve showing the effect of concentration of salts on the number of days to come up from seeds of various kinds. Average for sodium chlorid, sodium carbonate, and sodium sulphate.

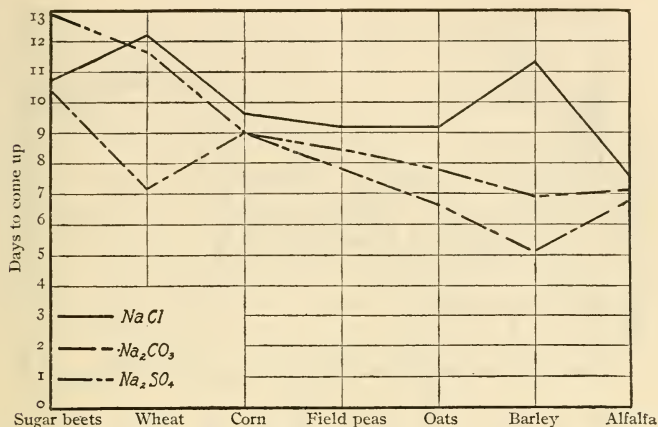


FIG. 40.—Curve showing the effect of sodium chlorid, sodium carbonate, and sodium sulphate on the number of days to come up from seeds of various kinds. Average for concentrations from 1,000 to 10,000 p. p. m.

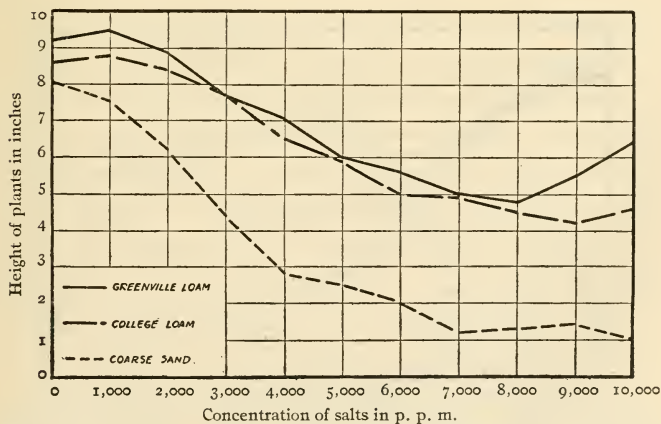


FIG. 41.—Curve showing the height of wheat plants germinating in College loam, Greenville loam, and sand with different concentrations. Average of 13 salts.

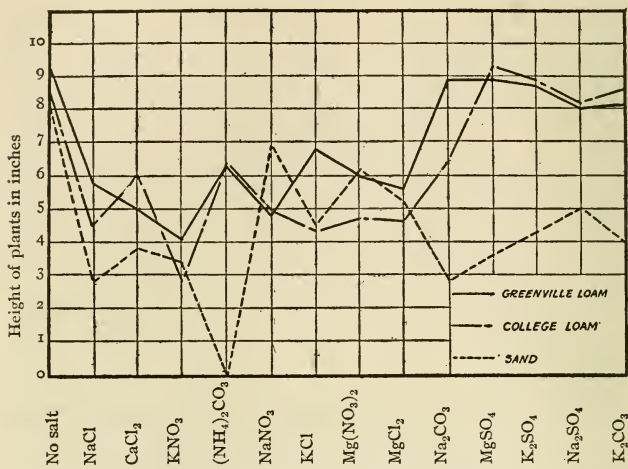


FIG. 42.—Curve showing the height of wheat plants germinating in College loam, Greenville loam, and sand containing various salts. Average for all concentrations.

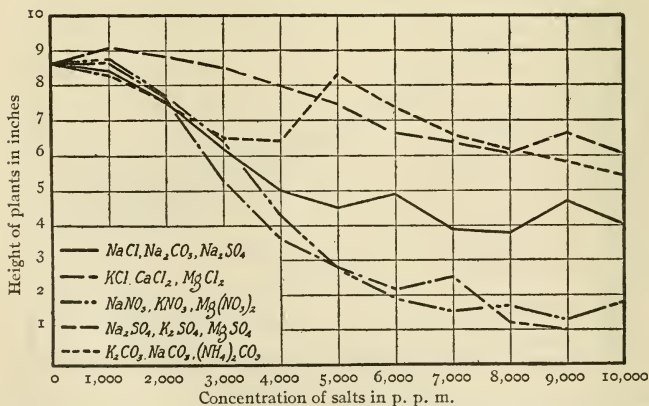


FIG. 43.—Curve showing the effect of various combinations of salts in different concentrations on the height of plants. Average of 15 combinations of each group of 3 salts.

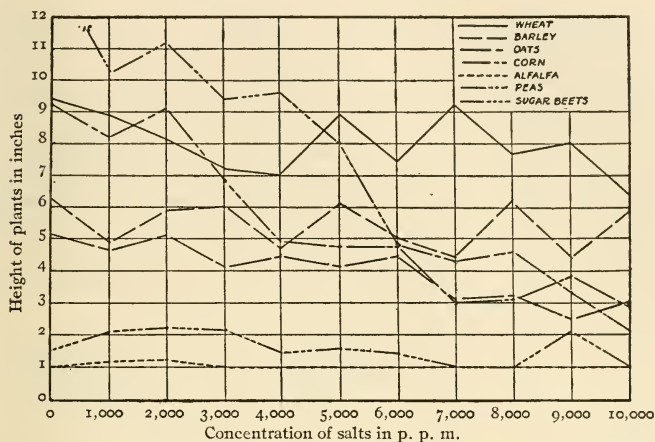


FIG. 44.—Curve showing the effect of concentration of salts on the height of plants from seeds of various kinds. Average for sodium chlorid, sodium carbonate, and sodium sulphate.

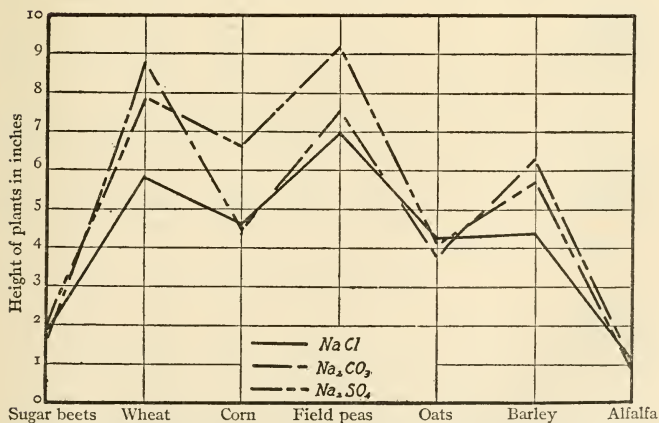


FIG. 45.—Curve showing the effect of sodium chlorid, sodium carbonate, and sodium sulphate on the height of plants from seeds of various kinds. Average for concentrations from 1,000 to 10,000 p. p. m.

ACTION OF THE VARIOUS IONS

COMPARISONS OF CATIONS AND ANIONS

In order to determine the effect of the different ions and to compare the relative action of the cations and anions, the results of the various tests were summarized and are presented in Tables XIII and XIV. These data represent the averages of the various concentrations of the salts in three different soils; hence, they should be fairly reliable.

On examining Table XIII it will be seen that the chlorid was by far the most toxic anion, followed by the nitrate, carbonate, and sulphate in the order named. This order held for all salts regardless of the basic ion, and is contrary to ideas on the subject previously held, as the carbonate was thought by many writers to be most injurious.

TABLE XIII.—*Effect of various anions on the germination and growth of wheat. Average for 3 soils and 10 concentrations for each soil*

Ions.	Number of trials.	Number of plants germinated.	Days to come up.	Average height of plants.	Average number of leaves per plant.	Weight of dry matter per glass.
				Inches.		Gm.
Sodium—						
Chlorid.....	30	2.3	11.2	4.3	1.35	0.020
Sulphate.....	30	7.0	9.0	7.0	1.77	.101
Carbonate.....	30	6.2	7.7	5.9	1.67	.072
Nitrate.....	30	3.3	8.6	5.5	1.62	.035
Average of sodium salts.	120	4.7	9.1	5.7	1.60	.057
Potassium—						
Chlorid.....	30	3.1	11.6	5.2	1.54	.040
Sulphate.....	30	7.1	6.5	7.3	1.75	.101
Carbonate.....	30	6.4	5.8	6.9	1.61	.087
Nitrate.....	30	3.7	9.0	3.4	1.29	.074
Average of potassium salts.....	120	5.1	8.5	5.7	1.55	.076
Magnesium—						
Chlorid.....	30	3.4	12.8	5.1	1.49	.039
Sulphate.....	30	7.9	6.7	7.3	1.72	.105
Carbonate.....	30	4.6	8.8	5.6	1.63	.052
Average of magnesium salts.....	90	5.3	9.4	6.0	1.61	.065
Calcium—						
Chlorid.....	30	2.8	12.1	4.9	1.66	.031
Ammonium—						
Carbonate.....	30	3.3	6.0	4.2	1.17	.044

In Table XIV a comparison is made of the various cations. Sodium is seen to be most injurious of all the bases except ammonium. Sodium is followed by calcium, potassium, and magnesium in the order named. This same order of toxicity held with all the acid radicals that were tried.

TABLE XIV.—*Effect of various cations on germination and growth of wheat. Average for 3 soils and 10 concentrations for each soil*

Cations.	Number of trials.	Number of plants germinated.	Days to come up.	Average height of plants.	Average number of leaves per plant.	Weight of dry matter per plant.
				<i>Inches.</i>		<i>Gm.</i>
Chlorid—						
Sodium	30	2.3	11.2	4.3	1.35	.020
Potassium	30	3.1	11.6	5.2	1.54	.040
Calcium	30	2.8	12.1	4.9	1.66	.031
Magnesium	30	3.4	12.8	5.1	1.49	.039
Average of chlorids	120	2.9	11.9	4.9	1.51	.033
Sulphate—						
Sodium	30	7.0	9.0	7.0	1.77	.101
Potassium	30	7.1	6.5	7.3	1.75	.101
Magnesium	30	7.9	6.7	7.3	1.72	.105
Average of sulphates	90	7.3	7.4	7.2	1.75	.102
Carbonate—						
Sodium	30	6.2	7.7	6.0	1.67	.071
Potassium	30	6.4	6.8	6.9	1.61	.087
Ammonium	30	3.3	6.0	4.2	1.17	.044
Average of carbonates	90	5.3	6.8	5.7	1.48	.067
Nitrate—						
Sodium	30	3.3	8.6	5.5	1.62	.035
Potassium	30	3.9	9.0	3.4	1.29	.074
Magnesium	30	4.6	8.8	5.6	1.63	.052
Average of nitrates	90	3.9	8.8	4.8	1.51	.054

A comparison of the various data presented in Tables XIII and XIV brings out clearly the fact that the injurious effects of the alkali salts in soils may be attributed more to the anion, or acid radical, than to the cation, or basic radical. All the chlorids gave results very similar to each other. The same may be said of the sulphates and nitrates. The different salts of sodium or potassium, on the other hand, differed greatly, according to the acid radical combined with them. This is just opposite to the conclusions of Kearney and Cameron (13) based on solution cultures.

RELATION OF MOLECULAR WEIGHT IN TOXICITY

A number of workers have considered the toxicity of various alkali salts to be proportional to their osmotic pressure. In order to determine whether this were true, the different salts which had been tested were arranged in the order of their toxicity and the molecular weight of each placed opposite to ascertain whether there was any relation between the two. Of course, it is understood that the lower the molecular weight of a salt the more molecules there are in a solution containing a given per-

centage of salt, and the more molecules there are the greater will be the osmotic pressure, provided there is the same dissociation. Following out this reasoning, a salt of low molecular weight should be more toxic than one of higher molecular weight if the salts were present in the same percentage by weight. Indeed, in the study of osmosis, salts would not be expressed in percentages but in molecular solutions. In soils, however, it is impossible to express salts on a basis of molecular solution.

In Table XV it will be seen that in a general way salts with low molecular weights are more toxic than those having a higher molecular weight, but there are so many exceptions that this can not be considered a general law holding for all salts. For example, magnesium sulphate has a lower molecular weight than potassium sulphate, sodium sulphate, potassium carbonate, or magnesium nitrate, and yet it is less toxic than any of these salts.

TABLE XV.—*Comparison of the toxicity of the various salts with their molecular weight*

Salts in order of toxicity.	Number of plants germinated.	Weight of dry matter produced.	Molecular weight.
		Gm.	
Sodium chlorid.	2. 3	0. 020	58. 5
Calcium chlorid.	2. 8	. 031	111. 0
Potassium chlorid.	3. 1	. 040	74. 6
Sodium nitrate.	3. 3	. 035	85. 1
Ammonium carbonate.	3. 3	. 044	202. 2
Magnesium chlorid.	3. 4	. 039	95. 3
Potassium nitrate.	3. 9	. 074	101. 2
Magnesium nitrate.	4. 6	. 052	148. 4
Sodium carbonate.	6. 2	. 071	106. 1
Potassium carbonate.	6. 4	. 087	138. 3
Sodium sulphate.	7. 0	. 101	142. 2
Potassium sulphate.	7. 1	. 101	174. 4
Magnesium sulphate.	7. 9	. 105	120. 4

SALTS ALONE AND IN COMBINATION WITH OTHER SALTS

One of the most important questions arising in connection with the toxicity of alkali is regarding the action of salts when present alone and when in combination with other salts. Considerable work has been done on the antagonistic action of various salts in solution cultures, and some very remarkable results have been obtained; but many of these results do not hold when the salts are applied to the soil.

An examination of figures 2 to 24 will show that in the soil the antagonistic action of the various alkali salts is not so great as previous workers have found for these same salts in solutions. For example, the magnesium salts when used alone in solution are very toxic to plants, but this is largely overcome by the presence of other salts. The results for mag-

nesium salts in soils do not show them to be particularly toxic. This is probably due in part to the high lime content of the soils used.

An attempt is made in Table XVI to bring together a summary of results for salts applied to soil singly and in combination. These are grouped as sulphates, carbonates, nitrates, chlorids, and the sodium salts. Under each salt are given certain figures which, when multiplied by 1,000, give the parts per million of salt added to the soil. Each figure is the average for Greenville loam, College loam, and sand. The results include the number of plants germinating in each glass, the weight of dry plant material produced in each glass, the average height of plants, and the average number of days required for the plants to come up.

TABLE XVI.—Effect of combination of salts on the germination and growth of wheat. Average of three soils

Figures under salts multiplied by 1,000 equal parts per million of salt in the soil

[illegible]

3	$\frac{1}{2}$	$\frac{1}{2}$	6.3	0.066	6.3	8.3	8.3	$\frac{1}{2}$	6	3.0	0.030	2.4	18.0	3	$\frac{1}{2}$	6.2	0.012	8.16	5	$\frac{1}{2}$	3	$\frac{1}{2}$	6.2	0.023	8.8	13.5
$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	6.3	0.070	6.3	0.088	8.3	$\frac{1}{2}$	6	3.0	0.033	2.4	18.0	3	$\frac{1}{2}$	6.2	0.023	8.16	5	$\frac{1}{2}$	3	$\frac{1}{2}$	6.2	0.023	8.8	13.5
$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	6.3	0.071	6.3	0.089	8.3	$\frac{1}{2}$	6	3.0	0.033	2.4	18.0	3	$\frac{1}{2}$	6.2	0.023	8.16	5	$\frac{1}{2}$	3	$\frac{1}{2}$	6.2	0.023	8.8	13.5
3	0	$\frac{1}{2}$	6.3	0.072	6.3	0.090	8.3	$\frac{1}{2}$	6	3.0	0.033	2.4	18.0	3	$\frac{1}{2}$	6.2	0.023	8.16	5	$\frac{1}{2}$	3	$\frac{1}{2}$	6.2	0.023	8.8	13.5
3	0	3	6.3	0.073	6.3	0.091	8.3	0	6	3.0	0.036	2.4	18.0	3	0	6.2	0.023	8.16	5	0	3	0	6.2	0.023	8.8	13.5
3	0	3	6.3	0.081	7.8	0.5	7.0	0	6	3.0	0.036	2.4	18.0	3	0	6.2	0.023	8.16	5	0	3	0	6.2	0.023	8.8	13.5
3	0	3	6.3	0.083	7.8	0.5	7.0	0	6	3.0	0.036	2.4	18.0	3	0	6.2	0.023	8.16	5	0	3	0	6.2	0.023	8.8	13.5
3	0	3	6.3	0.084	7.8	0.5	7.0	0	6	3.0	0.036	2.4	18.0	3	0	6.2	0.023	8.16	5	0	3	0	6.2	0.023	8.8	13.5
3	0	3	6.3	0.085	7.8	0.5	7.0	0	6	3.0	0.036	2.4	18.0	3	0	6.2	0.023	8.16	5	0	3	0	6.2	0.023	8.8	13.5
3	0	3	6.3	0.086	7.8	0.5	7.0	0	6	3.0	0.036	2.4	18.0	3	0	6.2	0.023	8.16	5	0	3	0	6.2	0.023	8.8	13.5
3	0	3	6.3	0.087	7.8	0.5	7.0	0	6	3.0	0.036	2.4	18.0	3	0	6.2	0.023	8.16	5	0	3	0	6.2	0.023	8.8	13.5
3	0	3	6.3	0.088	7.8	0.5	7.0	0	6	3.0	0.036	2.4	18.0	3	0	6.2	0.023	8.16	5	0	3	0	6.2	0.023	8.8	13.5
3	0	3	6.3	0.089	7.8	0.5	7.0	0	6	3.0	0.036	2.4	18.0	3	0	6.2	0.023	8.16	5	0	3	0	6.2	0.023	8.8	13.5
3	0	3	6.3	0.090	7.8	0.5	7.0	0	6	3.0	0.036	2.4	18.0	3	0	6.2	0.023	8.16	5	0	3	0	6.2	0.023	8.8	13.5
.....	9.3	1.109	6.7	7.3	0	0	6	3.3	0.073	2.5	12.8	0	0	6.3	0.032	2.715	2	0	6	0	6.0	0.0	0	0
4	2	2	8.4	0.069	8.8	6.2	4	2	8	0	0	0	0	0	2	8.0	0	0	0	4	2	2	8.4	0.024	8.5	13.5
2	2	2	8.5	0.074	9.4	6.8	2	4	8	7	0.091	1.0	18.0	2	4	8.1	0	0	0	2	4	2	8.4	0.024	8.5	13.5
2	2	4	8.6	0.081	9.4	6.8	2	4	8	7	0.092	2.12	0	2	4	8.1	0.020	0	0	2	4	2	8.0	0	0	0
2	0	0	8.6	0.054	8.0	8.0	2	0	8	1.3	0.041	1.0	18.0	2	0	8.0	0	0	0	2	0	2	8.0	0.080	5.2	9.0
0	0	0	8.7	0.104	7.0	8.0	0	2	8	0	0.15	2.4	18.0	0	2	8.0	0	0	0	2	0	2	8.0	0	0	0
0	2	0	8.7	0.088	9.2	8.4	0	2	8	7	0.092	2.0	12.0	0	2	8.0	0	0	0	2	0	2	8.5	0.041	8.4	13.5
0	0	0	8.5	0.079	9.2	8.4	0	2	8	7	0.091	0.02	12.0	0	2	8.0	0	0	0	2	0	2	8.0	0.025	10.2	2
0	2	0	8.6	0.094	8.3	8.9	0	2	8	7	0.091	1.0	12.0	0	2	8.0	0	0	0	2	0	2	8.0	0.043	8.4	12.8
2	0	0	8.6	0.102	6.9	9.4	2	0	8	1.7	0.061	1.1	18.0	2	0	8.0	0	0	0	2	0	2	8.0	0.021	10.14	0
4	4	0	8.3	0.058	8.8	8.4	4	0	8	0	0	0	0	0	4	8.0	0	0	0	4	0	4	8.0	0.075	8.1	10.2
4	0	4	8.3	0.095	6.7	9.2	4	4	8	7	0.039	3.7	15.1	0	4	8.0	0.003	1.0	18.0	0	4	4	8.0	0.03	5.1	13.5
4	0	4	8.3	0.086	6.4	9.4	4	0	8	0	0.038	5.7	11.9	4	0	8.0	0.002	2.5	18.0	4	0	4	8.0	0.011	10.13	5
0	0	0	8.6	0.075	7.8	10.0	0	4	8	0	0	0	0	0	4	8.0	0	0	0	4	0	4	8.0	0.073	8.8	12.4
0	0	0	8.6	0.103	9.2	8.0	0	0	8	0	0.054	7.7	5.5	0	8	8.0	0.004	1.0	12.0	0	8	0	8.0	0.058	5.5	7.0
0	0	0	8.7	0.108	9.2	8.4	0	0	8	0	0.064	7.7	14.3	0	8	8.0	0.004	1.0	12.0	0	8	0	8.0	0	0	0
5	$\frac{1}{2}$	$\frac{1}{2}$	10.3	0.068	8.1	8.2	5	$\frac{1}{2}$	10	0	0	0	0	5	$\frac{1}{2}$	10.0	0	0	0	5	$\frac{1}{2}$	$\frac{1}{2}$	10.2	0.012	10.13	5
$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	10.3	0.078	8.5	8.0	$\frac{1}{2}$	$\frac{1}{2}$	10	0	0	0	0	$\frac{1}{2}$	$\frac{1}{2}$	10.0	0	0	0	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	10.3	0.012	10.13	5
$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	10.3	0.079	8.1	7.8	$\frac{1}{2}$	$\frac{1}{2}$	10	0	7	0.001	1.0	12.0	5	10.0	0	0	0	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	10.0	0	0	0
5	0	5	10.3	0.086	8.0	7.0	5	0	10	0	0	0	0	5	0	10.0	0	0	0	5	0	5	10.5	0.050	8.0	13.5
5	0	5	10.3	0.085	8.1	7.8	5	0	10	0	7	0.001	1.0	12.0	5	10.0	0	0	0	5	0	5	10.0	0	0	0
5	0	5	10.3	0.086	8.1	7.8	5	0	10	0	7	0.001	1.0	12.0	5	10.0	0	0	0	5	0	5	10.0	0	0	0
5	0	5	10.3	0.087	8.1	7.8	5	0	10	0	7	0.001	1.0	12.0	5	10.0	0	0	0	5	0	5	10.0	0	0	0
10	0	10	10.3	0.084	7.5	8.0	10	0	10	0	0	0	0	10	0	10.0	0	0	0	10	0	10	10.0	0.038	8.0	13.5
10	0	10	10.3	0.085	7.7	8.2	10	0	10	0	0	0	0	10	0	10.0	0	0	0	10	0	10	10.0	0.038	8.0	13.5
10	0	10	10.3	0.086	7.9	7.1	8.2	10	0	10	0	0	0	10	0	10.0	0	0	0	10	0	10	10.0	0.038	8.0	13.5
0	0	0	10.4	0.084	9.5	5.4	0	0	10	0	0	0	0	0	0	10.0	0	0	0	0	0	0	10.0	0.038	8.0	13.5

The top line in each case gives the results where no salts were applied. Below this the figures are arranged according to the total quantity of salt used, first 1,000 p. p. m., followed by 2,000, 4,000, 6,000, 8,000, and 10,000. It will be noted that with the chlorids and nitrates practically no plants grew in the higher concentrations. Careful study of the table is necessary to see the numerous complex relations that are brought out between the various salts. The simple relations may be seen more easily in figures 2 to 24, but by bringing together a large mass of data in one table many relations can be found that could not be seen in the diagrams.

The average alkali of Utah contains a mixture of chlorids, sulphates, and carbonates, with the carbonates usually present only in small quantities. The practical alkali problem, therefore, is largely centered around the sulphates and chlorids of sodium. An examination of Table XVI does not seem to indicate that either of these salts has any great neutralizing effect on the other.

A general conclusion from this table might be that where alkali salts are found together in the soil the toxic action of the combined salts is only slightly less than the sum of the toxicities of the individual salts. It may be that with other combinations of salts this conclusion would not be justified.

PRACTICAL LIMITS OF THIS PROBLEM

The practical problem of this entire study is to determine the quantity of various alkali salts necessary in the soil to reduce the growth of crops beyond the point of profitable production. Under the conditions of dry farming there is no practicable way of removing excessive soluble salts; hence, if salts are found in these soils in quantities prohibiting crop growth, the soils are valueless for agriculture. On the other hand, soils that are susceptible of irrigation and drainage may be reclaimed by the leaching out of the alkali. In any soil, however, where there is a likelihood of alkali injury it is very important, in order to be able to judge the value of a soil, to know exactly how much of a given salt is necessary to injure crops. The literature on the subject up to the present is somewhat conflicting and lacks the definiteness that would be desirable.

There are so many factors entering into the toxicity of alkali that it is difficult to assign definite toxic limits. For example, an analysis might show a soil to contain a given percentage of salt when in reality the greater part of the salt might be in a crystallized form at the surface, where it would do no harm until dissolved and washed back into the soil. It is the salt in solution that does the real injury. The wetness of the soil, its texture, the presence of neutralizing substances, and a number of other factors all alter the toxicity of soluble salts, which

makes it impossible to say exactly what are the practical limits of alkalis.

In getting the limits given below it was considered that when alkali retarded germination and growth to about half what they were in soils without alkali the practical limit had been reached. Certainly it would not be profitable to use a soil where alkali decreased yields below half normal.

Figures 46 and 47 show the practical limits of growth of wheat in loam and sand for 13 different salts. It will be noted that these salts bear a similar relation to each other in both kinds of soil, although only about half as much alkali is required in sand to reach the toxic limit as in loam. One of the most striking features about the diagram is the fact that in sand the carbonates are proportionately more toxic when compared with other salts than they are in loam.

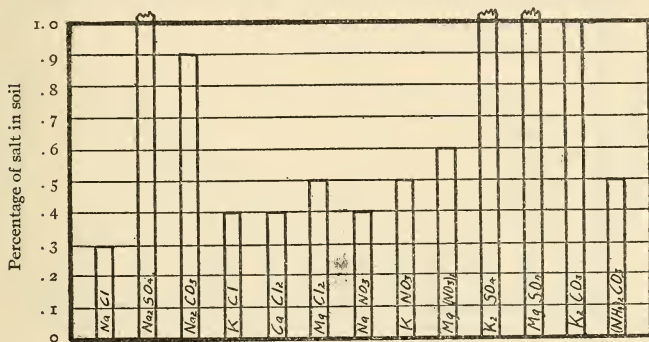


FIG. 46.—Diagram showing the percentage of alkali salt in loam soil giving about half normal germination and production of dry matter in wheat.

Loam having 0.3 per cent and sand having 0.2 per cent of sodium chlorid contain a limit of this salt for the profitable production of crops. The other chlorids may be somewhat higher, while the nitrates may be about 0.1 per cent higher than the chlorids. On loam crops grow well with as high as 1 per cent of the sulphates, while in sand from 0.5 to 0.7 per cent of the sulphates is injurious.

Figure 48 gives a comparison of the resistance of barley, oats, wheat, alfalfa, sugar beets, corn, and Canada field peas for sodium chlorid, sodium carbonate, and sodium sulphate in loam. Barley can withstand 0.5 per cent of sodium chlorid, 1 per cent of sodium carbonate, and more than 1 per cent of sodium sulphate. All crops in the test except oats, sugar beets, corn, and field peas produced more than half normal growth where 1 per cent of sodium sulphate was present. There was a great difference in the resistance of various crops to sodium carbonate, the

practical limit ranging from 0.4 per cent for Canada field peas up to 1 per cent for barley. Sodium chlorid showed about the same toxicity for all the crops except barley and oats, which were slightly more resistant. The striking point about this diagram is the fact that the relative toxicity of the different salts varies for each crop.

SUMMARY

(1) The effect of the various alkali salts in soils on plant growth and the quantity of alkali that must be present to injure crops are of great practical importance to farmers in arid regions, as well as of considerable interest to the scientist.

(2) A great amount of work has already been done on alkali, but this does not give all the information that is needed.

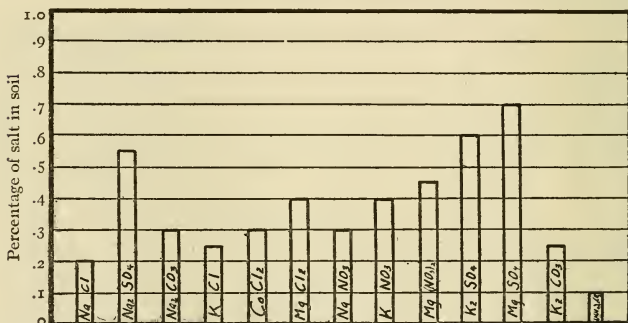


FIG. 47.—Diagram showing the percentage of alkali salt in coarse sand giving about half normal germination and production of dry matter in wheat.

(3) In this paper results of over 18,000 determinations of the effect of alkali salts on plant growth are reported.

(4) Only about half as much alkali is required to prohibit the growth of crops in sand as in loam.

(5) Crops vary greatly in their relative resistance to alkali salts, but for the ordinary mixture of salts the following crops in the seedling stage would probably come in the order given, barley being the most resistant: Barley, oats, wheat, alfalfa, sugar beets, corn, and Canada field peas.

(6) Results obtained in solution cultures for the toxicity of alkali salts do not always hold when these salts are applied to the soil.

(7) The percentage of germination of seeds, the quantity of dry matter produced, the height of plants, and the number of leaves per plant are all affected by alkali salts in about the same ratio.

(8) The period of germination of seeds is considerably lengthened by the presence of soluble salts in the soil.

(9) The anion, or acid radical, and not the cation, or basic radical, determines the toxicity of alkali salts in the soil. Of the acid radicals used, chlorid was decidedly the most toxic, while sodium was the most toxic base.

(10) The injurious action of alkali salts is not in all cases proportional to the osmotic pressure of the salts.

(11) The toxicity of soluble salts in the soil was found to be in the following order: Sodium chlorid, calcium chlorid, potassium chlorid, sodium nitrate, magnesium chlorid, potassium nitrate, magnesium nitrate,

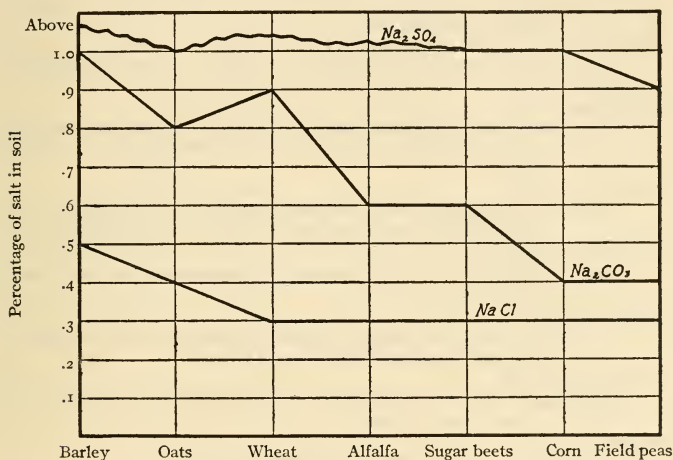


FIG. 48.—Curve showing the percentage of sodium chlorid, sodium carbonate, and sodium sulphate in Greenville loam giving about half normal germination and production of dry matter.

sodium carbonate, potassium carbonate, sodium sulphate, potassium sulphate, and magnesium sulphate.

(12) The antagonistic effect of combined salts was not so great in soils as in solution cultures.

(13) The percentage of soil moisture influences the toxicity of alkali salts.

(14) Salts added to the soil in the dry state do not have so great an effect as those added in solution.

(15) Land containing more than about the following percentages of soluble salt are probably not suited without reclamation to produce ordinary crops. In loam, chlorids, 0.3 per cent; nitrates, 0.4 per cent; carbonates, 0.5 per cent; sulphates, above 1.0 per cent. In coarse sand, chlorids, 0.2 per cent; nitrates, 0.3 per cent; carbonates, 0.3 per cent; and sulphates, 0.6 per cent.

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HISTOLOGICAL RELATIONS OF SUGAR-BEET SEEDLINGS AND *PHOMA BETAE*

By H. A. EDSON,¹

Physiologist, Office of Cotton and Truck Disease Investigations, Bureau of Plant Industry

In a former paper ² it was pointed out that practically all sugar-beet (*Beta vulgaris*) seed is more or less heavily infected with *Phoma betae* (Oud.) Fr., and that a large proportion of the seedlings developing from such stock suffer from incipient or severe attack of the fungus, but that under favorable conditions a high percentage of the attacked plants recover sufficiently to make a good growth. It appears that the period during which the sugar beet is susceptible to infection by this fungus is confined to the seedling stage, or, in the case of leaves, to old age, but that when infection has once occurred, it persists. After apparent recovery of the host, the fungus is still present, although it remains concealed until conditions arise sufficiently unfavorable to the beet to enable the parasite to renew its attack. Except in the seedling stage, it seldom accomplishes the immediate destruction of its host, but remains inactive during the first growing season and becomes destructive on mother beets in storage or reappears during the second growing season on the seed stalks or racemes in time to cause infection of the new crop of seed.

Histological studies recently conducted upon seedling sugar beets infected with *Phoma betae* have shown the fungus fruiting on the surface of young plants that were scarcely past the cotyledon stage. They have also revealed the organism living without serious injury to the host, within the deeper cells of plants that had thrown off the attack and which could safely be predicted to show no further sign of infection during the growing season if reasonably good cultural conditions were maintained. The slides show that the fungus may persist both in and on the tissues of the beet and also indicate something of its *modus operandi* in attack on seedlings. Sections were prepared from material grown from pasteurized seed in experimental pots in sterilized soil which had been inoculated at the time of seeding with pure cultures of the fungus. The material was controlled by check pots and by recovery of the fungus from certain of the seedlings from each pot as the disease appeared. Damped-off and root-sick seedlings selected at different stages in the progress of the disease and healthy

¹ The author wishes to acknowledge his indebtedness to Mrs. Nellie D. Morey, formerly of the Office of Cotton and Truck Disease Investigations, for assistance in the preparation of slides.

² Edson, H. A. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. *In Jour. Agr. Research*, v. 4, no. 2, p. 135-168, pl. 16-26. 1915.

seedlings from the control pots were killed in Flemming's solution, embedded, sectioned, and stained with the triple combination in the usual way. Camera-lucida drawings from the slides thus prepared are employed to illustrate this discussion. Most of the seedlings were still in the cotyledon stage, but some that had recovered from the attack had developed their first pairs of leaves. Seedlings which had been entirely killed were so badly disintegrated or so softened by the disease that they did not yield satisfactory material for study. The sections showed the cells in a condition of complete collapse and decay. The cellulose layers of the walls, as well as the middle lamella, were gelatinized and softened to such an extent as to have lost most of their rigidity. The walls were broken and fragmented, but this may have resulted from handling during the process of washing and dehydrating. Bacteria were present, of course, and the softening of the walls, which made them so liable to fracture in handling, may have been due in part to the action of these agents.

Cells of badly diseased but still living seedlings presented more favorable material for studying the histological relations of the parasite and host. The cells were often nearly filled with the fungus, which showed a tendency to remain within the cell rather than in the middle lamella, although it frequently penetrated the walls (Pl. I, fig. 1). Now and then a thread of the fungus was observed running between the cells for a little distance, but the indications are that, while the organism dissolves the middle lamella, it does not feed upon it. Heavily invaded cells are consumed, the cytoplasm disappears, and the nuclei disintegrate. The middle lamella gelatinizes, so that the cellulose lamellæ may become widely separated while the cellulose layers are broken and disintegrated or even dissolved (Pl. I, fig. 2). The first visible indication of the alteration in the walls is a change in their reaction toward the stain. They take the safranin more deeply and retain it more tenaciously than do the walls of normal cells. With the progress of the disease a border area of increasing width, which also takes the safranin deeply, develops on either side of the walls, as if the substances which retained the dye were gradually diffusing from the wall and spreading into the surrounding space.

In cases of less serious infection, where recovery is possible, or in tissues which have just been invaded, a somewhat different condition exists. Plate I, figure 3, represents a recently invaded portion of a rather badly diseased seedling which would probably have been unable to recover. The cell walls show the gelatinized condition only in a moderate degree and in an area confined to the points where it has been penetrated by the mycelium. The mycelium has expanded in one of the cells in a manner not frequently noted, and the effect of the parasitism is apparent in the abnormal condition of the host nuclei. Evidence of disease was sometimes manifested in the neighboring uninfected cells of such mate-

rial by the unusual appearance of the nuclei. Dumb-bell forms, budding, and indirect division were observed occasionally, but never in any large number (Pl. I, fig. 4, 5, 6).

The most interesting phenomena in many respects, as well as the most puzzling, are those associated with recovery and healing. Sugar beets attacked by the fungus frequently send out new side roots from a point above the invasion and succeed in preventing the destruction of this new growth. Cases were common in which the region invaded and disintegrated had been confined to the outer tissue. The central vascular region and the surrounding layers of cells resisted the attack and eventually succeeded in sloughing off the killed tissue. The fungus was frequently found developing its pycnidia on the killed portions of such recovering seedlings, while the host tissue, only a few cells below, appeared perfectly normal (Pl. II, fig. 1).

The most striking thing brought out by a study of the sections, however, is the presence of the fungus apparently established in a condition of reduced relative virulence in the interior tissue of beets which have recovered from the attack and which are assured of making a good growth (Pl. II, fig. 2). In such cases even the invaded cells are not killed, and the adjacent ones appear perfectly normal in every respect. So far as has been observed, the cells thus invaded are adjacent to vascular tissue, but the organism has never been seen in the conducting elements. The infection is confined to a vertical chain of cells, and in no case was more than a single unbranched hypha observed.

The physiological relation here presented is an exceedingly interesting one and its investigation is of the highest scientific and practical importance.

It is difficult to explain just how an organism capable of producing such complete collapse in cells of seedlings should suddenly find its action checked and confined to a saprophytic existence on an area of discarded surface tissue, but the means by which it establishes itself within the highly nutritive living cells of the interior and is at the same time compelled to remain in a quiescent condition is still more problematical. The condition presents a relatively highly developed type of parasitism in which the organism voluntarily or by compulsion permits the completion of the normal life history of the host while securing for itself the assurance of perpetuation through infection of the seed. The balance, however, is not a perfect one, since, if the host encounters sufficiently adverse conditions during either of the growing seasons or in storage, the activity of the parasite is renewed and the sugar beet is destroyed, thus preventing seed production and the perpetuation of the parasite through the seedling channel.

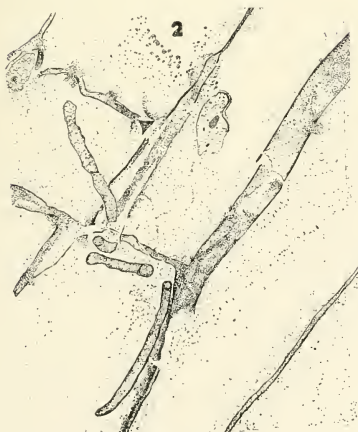
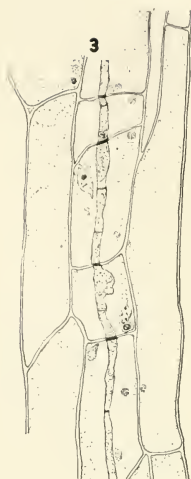
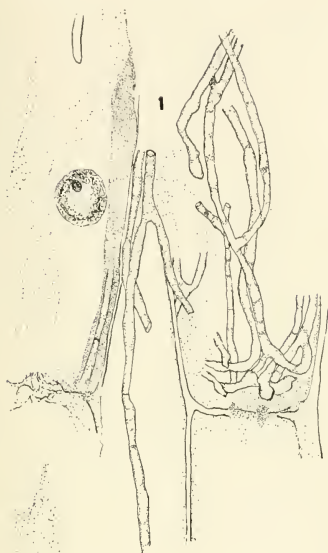
PLATE I

Fig. 1.—Section of a sugar-beet seedling invaded by *Phoma betae*, showing distribution of the mycelium and the action of the fungus on the protoplasm and cell walls. $\times 530$.

Fig. 2.—Section of sugar-beet seedling showing characteristic action of *Phoma betae* on the cytoplasm and nuclei and cell walls in cases of serious infection. Note the gelatinized condition of the middle lamella. $\times 530$.

Fig. 3.—Section of sugar-beet seedling showing *Phoma betae* penetrating the cell walls and expanding in one of the cells. The nuclei show signs of degeneration. $\times 530$.

Fig. 4, 5, and 6.—Abnormal nuclei from uninfected cells adjacent to invaded tissue of sugar-beet seedlings. The nucleus in figure 6 appears to be in the process of direct division. $\times 1,330$.



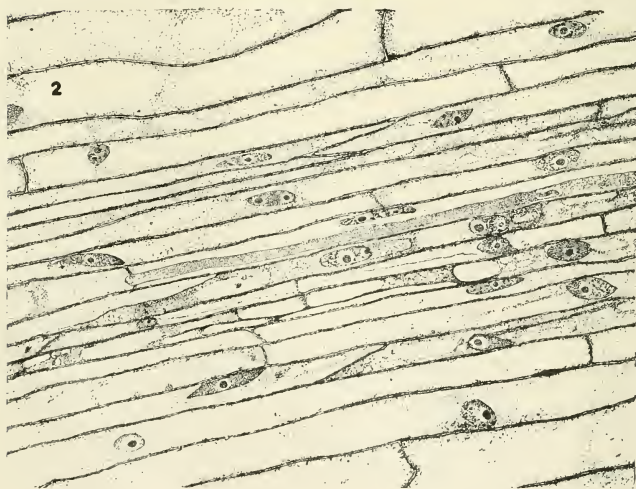


PLATE II

Fig. 1.—Section through a sugar-beet seedling which has recovered from an attack of *Phoma betae*, showing a young pycnidium of the fungus forming on the discarded, killed tissue. $\times 500$.

Fig. 2.—Longitudinal section through a sugar-beet seedling which had recovered from an attack of root sickness due to *Phoma betae*, showing the presence of the fungus established in a condition of reduced virulence in the living cells. $\times 530$.

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PERENNIAL MYCELIUM IN SPECIES OF PERONOSPORA RACEAE RELATED TO PHYTOPHTHORA INFES- TANS

By I. E. MELHUS,

Pathologist, Cotton and Truck Disease Investigations,
Bureau of Plant Industry

INTRODUCTION

Phytophthora infestans having been found to be perennial in the Irish potato (*Solanum tuberosum*), the question naturally arose as to whether other species of Peronosporaceae survive the winter in the mycelial stage. As shown in another paper (13),¹ the mycelium in the mother tuber grows up the stem to the surface of the soil and causes an infection of the foliage which may result in an epidemic of late-blight.

Very little is known about the perennial nature of the mycelium of Peronosporaceae. Only two species have been reported in America: *Plasmopara pygmaea* on *Hepatica acutiloba* by Stewart (15) and *Phytophthora cactorum* on *Panax quinquefolium* by Rosenbaum (14). Six have been shown to be perennial in Europe: *Peronospora schachtii* on *Beta vulgaris* and *Peronospora dipsaci* on *Dipsacus follonum* by Kühn (7, 8); *Peronospora alsinearum* on *Stellaria media*, *Peronospora grisea* on *Veronica hederaefolia*, *Peronospora effusa* on *Spinacia oleracea*, and *Atriplex hortensis* by Magnus (9); and *Peronospora viticola* on *Vitis vinifera* by Istvanffi (5).

Many of the hosts of this family are annuals, but some are biennials, or, like the Irish potato, are perennials. Where the host lives over the winter, it is interesting to know whether the mycelium of the fungus may also live over, especially where the infection has become systemic and the mycelium is present in the crown of the host plant. The absence or sparse production of oospores in some of the species of Peronosporaceae, coupled with the appearance of the fungus as soon as the host puts out foliage in the spring, suggests that the mycelium may play an important

¹ Reference is made by number to "Literature cited," p. 68-69.

rôle in bridging over the winter. This paper gives the results of experiments and observations which show that in the Northern States species of the *Peronosporaceae* which have perennial mycelium are common and that the mycelium may live from one growing season to another in the living diseased host tissues.

In several of these experiments the locality where infected plants were growing was marked in the autumn and the plants collected from time to time during the winter and early spring, after which they were allowed to revive in the greenhouse and a careful watch kept for any evidence of fruit of the fungus. In other cases the underground parts of infected plants were taken in the spring and planted in steam-sterilized soil in the greenhouse, and when the shoots came through the ground conditions were made favorable for the sporulation of the fungus. In still other cases the presence of the mycelium in perennial parts of the host was determined microscopically.

PERONOSPORA PARASITICA

Late in the fall of 1910 and 1911 it was observed that young plants of *Lepidium virginicum* in the vicinity of Madison, Wis., were very generally infected with *Peronospora parasitica* and that the tissues of these plants contained few or no oospores, although they were produced in abundance in the summer when the host tissues were dying. Plants of *Lepidium* sp. always form a rosette of leaves in the late fall, and some of these remain alive through the winter.

In the fall of 1911 two patches of *Lepidium* plants, about 50 per cent of which were infected with *Peronospora parasitica*, were marked so that they might be easily found during the winter. One was on the side of a short incline made by dumping several loads of soil in a heap and the other on the parking of a city drive in Madison. Both patches were well exposed during the winter of 1911-12, which was unusually severe, there being no covering of snow on the former at any time and the latter being covered only a part of the time.

After the first killing frost, which, according to the Weather Bureau, occurred on October 24, infected plants of *Lepidium virginicum* were collected at various times during the winter. Beginning on October 30, a test was made of the germination of the conidia of *Peronospora parasitica* growing on *Lepidium virginicum*. Although when alive the conidia of this fungus usually germinate profusely within 2 to 3 hours and always within 24 hours, no germination occurred in this test, although exposed to favorable conditions for 48 hours. This coincides with what is known of the behavior of the spores of other species—e. g., *Cystopus candidus* (Melhus, 10)—and excludes the possibility of these conidia becoming a source of further infection. A careful search for oospores was made after October 30 in a large number of infected plantlets, but none was found.

The first collection of plants of *Lepidium virginicum*, numbering about 20, was made on November 5, enough soil being taken up with each plant to keep the roots from being disturbed. The plants were taken to the greenhouse and transplanted in two flats, or shallow boxes, and on November 6 each box was covered with a low bell jar to keep the air moist, a condition favorable for the sporulation of the fungus. An examination of the plants next day showed but 2 inactive, the leaves of the other 18 being turgid and expanded in the normal way. It also showed that 2 of the plants were covered with a white glistening growth, which on microscopic examination was found to be the spores and conidiophores of *Peronospora parasitica*. The following day this fungus was found sporulating on 3 additional plants, and 8 days after the plants had been collected it was found fruiting on some portion of 12 of the 18 living. Although kept under observation for 6 weeks, the remaining six plants were free from infection, which showed that it did not take place under the conditions in which they were held in the greenhouse.

On December 14 another collection of plants of *Lepidium virginicum* was taken from the patch on the parking near the drive, the soil at that time being frozen 6 inches deep. A block of soil on which there were 18 of the plants was chopped loose and placed in a flat in the greenhouse, and after being allowed to thaw out for 24 hours was covered with a glass house. On December 17, 3 days after the plants were brought into the greenhouse, 1 was nearly covered with conidiophores and spores of *Peronospora parasitica*, the next day 4 more showed fruit of the fungus, and at the end of the sixth day an additional plant, or 6 in all, showed fruiting of the fungus, indicating that at least that number was infected when collected (Pl. III, fig. 2, A). The fungus fruited on both sides of the leaves and also on the new leaves developing from the crown, though not as abundantly on these as on the older leaves.

Besides the collections of November 5 and December 14, 4 others, or a total of 102 plants, were brought into the greenhouse from the 2 patches during the dormant period of the host plant. In the case of several of these collections *Peronospora parasitica* sporulated on some of the plants 2 days after their transfer to the greenhouse, but usually the disease did not appear before 3 to 5 days and, when the infection was weak, not before 8 days after the transfer. Table I gives date of collection, number of plants in each collection, date of first evidence of *Peronospora*, number of days required for the fungus to sporulate, and number of plants on which the disease appeared.

TABLE I.—Record of six collections of plants of *Lepidium virginicum* infected with *Peronospora parasitica*

Date of collection.	Number of plants.	Date of sporulation.	Number of days required for sporulation.	Number of plants on which fungus sporulated.
1911				
Nov. 5.....	20	Nov. 8	3	12
Dec. 14.....	18	Dec. 17	3	6
Dec. 18.....	12	Dec. 20	2	6
1912.				
Feb. 22.....	11	Feb. 27	5	1
Mar. 6.....	24	Mar. 10	4	7
Mar. 25.....	17	Mar. 27	2	9

As shown by Table I, 41 plants, or about 40 per cent of the collections, were infected before their transfer to the greenhouse.

It might be supposed that oospores produced the previous year were in the soil immediately around and adhering to the plants collected and that when warmed up in the greenhouse these germinated and produced the infections noted. To test this possibility, 25 leaves were collected from the plants in the two patches, washed very thoroughly in running water, and placed in a moist chamber, while 25 other leaves were collected from the same plants, and without being washed were placed under similar conditions as controls. In both cases the fungus sporulated after three days, and, although much less than when the leaves were on the plant, the sporulation produced sufficient conidiophores to be plainly visible to the naked eye, a growth which could probably not be produced by oospores.

Besides this evidence that *Peronospora parasitica* renews itself by means of mycelium as well as oospores, the writer failed to germinate oospores after repeated attempts. He has also shown (11) that *Peronospora parasitica* on *Lepidium virginicum* can be collected at any time during the winter and early spring, brought into the greenhouse, and made to fruit. Moreover, there can be no doubt that the sporulation on the plant collections at Madison was due to living mycelium in the host tissue.

CYSTOPUS CANDIDUS

Lepidium virginicum is attacked not only by *Peronospora parasitica* but also by *Cystopus candidus*, a fungus which can undoubtedly propagate itself from year to year by mycelium remaining dormant in the living host tissues through the winter. As is well known, these two fungi often infect a plant simultaneously, as was the case of some of the plants from the parking near the drive. In the collections made on December 14, 1911, one plant showed white pustules of *Cystopus candidus* on December 17, three days after the plants were collected. The following day

two additional plants showed white pustules of this fungus and also spores of *Peronospora parasitica*, the number of pustules increasing on the lower side of the leaves until many were well spotted. Two plants in the collection made on February 22 bore white pustules within three days after they were taken into the greenhouse, showing that they were infected with *Cystopus candidus* and that the fungus was alive in the tissues in late winter (Pl. III, fig. 1). Again, in the collection made on March 25 one plant developed pustules of *Cystopus candidus* and conidio-phores and spores of *Peronospora parasitica* four days after being transferred to the greenhouse.

Cystopus candidus is also a very common parasite on *Capsella bursa pastoris*, a plant that may become a winter annual. In the fall of 1911 a patch of plants of *Capsella bursa pastoris*, many of which were infected with *Cystopus candidus*, was marked; and on March 30, 1912, 25 plants were collected and treated in the same way as the plants of *Lepidium virginicum* infected with *Peronospora parasitica*. After two days the plants began to show signs of life; and at this time they were covered with a small glass house. Three days later white pustules were discovered on one leaf; and the following day, or six days after the plants were brought in, white pustules developed on other leaves of the same plant.

On April 5, 1912, just as the ground thawed out, another collection, consisting of 76 plants, was made. Four days after, or on April 9, there were white pustules on four of the plants. Except in the case of one large leaf, which was probably produced early the preceding fall, the pustules were all on the youngest leaves, which indicates that the mycelium can winter over in leaves of plants of *Capsella bursa pastoris* that live through the winter. The fact that the youngest leaves were infected suggested crown infection; and later this proved to be the case, all of the leaves growing from certain plants being infected as soon as they appeared, while the leaves growing from certain others remained free from infection. On April 10 white pustules appeared on two other plants, making a total of six infected plants in the second collection. As soon as the plants of *Capsella bursa pastoris* in the marked patch started to grow in the spring some of them showed infection with *Cystopus candidus*, which developed like the infections studied in the greenhouse. From these experiments it will be seen that the mycelium of *Cystopus candidus* in the tissues of the host remains alive through the winter.

In the fall of the year *Cystopus candidus* becomes systemic in the tissues of *Sisymbrium officinale* and *Brassica nigra* also. So far these two host plants have not been followed through from fall to spring, but, like the plants of *Lepidium virginicum* and *Capsella bursa pastoris*, both may become winter annuals, as is well known.

PERONOSPORA FICARIAE

On May 10, 1911, *Peronospora ficariae* was very prevalent on *Ranunculus fascicularis* in the vicinity of Madison. This fact, coupled with De Bary's (3) statement in connection with his discussion of the perennial nature of mycelium of *Phytophthora infestans*, that *Peronospora ficariae* is perennial in the tissues of *Ranunculus ficaria*, led the writer to determine whether it survives the winter in the mycelial stage on *Ranunculus fascicularis* also. Eighteen plants, very generally infected with the disease, were staked on the date above mentioned so that they could be readily located throughout the winter and following spring. On February 2, 1912, five of the plants were chopped out of the frozen ground and carried into the greenhouse, where the adhering soil was allowed to thaw out and was removed from the fascicled roots, after which the roots were carefully washed until free from soil and then transplanted in greenhouse soil. The plants, two of which refused to grow, started very slowly, the first one coming up on March 3, and two others the following day. The young plants were chlorotic, distorted, and yellowish green, but there was no evidence of *Peronospora ficariae* present until they had been held under small bell jars for 24 hours, after which the fungus present on the deformed leaves fruited profusely, showing plainly that the fungus was alive in the host tissues during the winter.

The 13 plants that were left in the marked space from which the 5 were taken were also watched carefully after they began to come up. On April 5 five appeared, and these were covered with small bell jars. On the following day conidiophores and spores of *Peronospora ficariae* were collected from the underside of the leaves, showing that in this case also the plants were infected before they reached the surface of the soil. The results of these experiments confirm De Bary's (3) statement and also show that *Peronospora ficariae* is perennial not only in *Ranunculus ficaria* but also in *Ranunculus fascicularis*.

PERONOSPORA VICIAE

Peronospora viciae occurs on several of the legumes. On May 11, 1913, the writer found it to be quite abundant on *Vicia sepium*, a perennial common in the District of Columbia. At that time about 25 per cent of the plants, which were from 4 to 6 inches high, were infected with the disease, the fungus sporulating profusely and the plants giving every evidence of systemic infection. The location of these plants was staked off on the date above mentioned and the patch kept under observation. On April 5 the following spring the plants started to come up, the tallest being only 2 inches, and at this early stage nine were found to be systemically infected. It was not uncommon to find a healthy and a diseased plant within 2 inches of each other. If infection was caused by oospores or conidia, it is difficult to understand why the infection was not general

in the patch and why plants growing near each other should be infected in some cases and not in others.

As the host is a perennial, as infection by *Peronospora viciae* is systemic, and as oospores are produced only sparingly, if at all, on *Vicia sepium*,¹ it seems very probable that the mycelium survives the winter in the living tissues of the host.

PLASMOPARA HALSTEDII

In the spring of 1911 *Plasmopara halstedii* was found to be very abundant on some young plants of *Helianthus diversicatus* about 6 inches high. The plants were somewhat dwarfed, chlorotic, and well covered with conidiophores, giving every evidence of systemic infection. The location of the infected plants was marked and observations made during the winter and spring of 1912.

Fourteen of the plants that were very generally infected were staked, and on January 4, three of these were chopped out of the ground and transplanted in the greenhouse in exactly the same way as were the *Lepidium* plants infected with *Peronospora parasitica*. Each of these rhizomes produced a chlorotic shoot which was covered with spores of *Plasmopara halstedii*. On March 4 four more were brought into the greenhouse. One of these rotted in the soil, but each of the others produced a shoot, which showed infection as soon as it appeared above ground.

The remaining seven of the fourteen staked were left in the patch and kept under observation. On May 10, when they were 3 to 6 inches high, all were found to be infected with *Plasmopara halstedii*, except one plant, which was entirely free from infection, as were many others in the immediate vicinity. Two of these plants were now dug up, and portions of the stems at their junction with the rhizomes were fixed in various strengths of Flemming's killing fluid. Paraffin sections cut from this material and stained showed abundant mycelium in all parts of the stem except the fibrovascular bundles, the mycelium being entirely intercellular with globular haustoria extending into the cells, as shown in figure 1. The presence of the mycelium in the stem at its junction with the rhizome shows that the infection was systemic and probably came from the rhizome in the beginning.

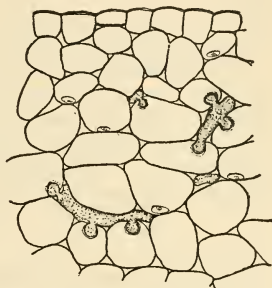


FIG. 1.—A cross section of a stem of *Helianthus diversicatus* which is infected with *Plasmopara halstedii*. The mycelium is shown in the cortex at the junction of the stem with the rhizome of the host.

¹ The writer searched many times in the tissues of all stages of maturity for resting spores, but without success.

The remaining five of these seven infected plants were carefully dug up, the stems cut off at their junction with the rhizomes, washed very clean with a brush, and disinfected in corrosive sublimate for five minutes. After this they were planted in steam-sterilized soil in the greenhouse, in which there had never been any *Plasmopara halstedii*. On May 23 two shoots broke through the ground; and three days later, when one was 1 inch and the other 2 inches high, they were covered with jelly glasses in order to keep the atmosphere moist. On this date the initial leaves appeared chlorotic, but no spores of *Plasmopara halstedii* could be found. The next day the lower surfaces of the leaves were almost covered with a glistening white coat of conidiophores and spores, which on microscopic examination were found to be the conidia of *Plasmopara halstedii*. Of the three remaining rhizomes, two failed to come up, while the third sent up a spindly shoot on June 5. This shoot was treated in the manner already described and the fungus fruited in the same way.

This experiment showed that the diseased plants grown in the greenhouse manifested the same symptoms as those grown in the open. It also showed that the mycelium of *Plasmopara halstedii* may be present in the rhizome of *Helianthus diversicatus*, and this, coupled with the observations described, strongly suggests that *Plasmopara halstedii* is perennial in the rhizomes of *Helianthus diversicatus*.

CONCLUSIONS

As seen from these investigations, several species of the Peronosporaceae live over from one growing season to another by at least two means: Resting spores and perennial mycelium. As is well known from the excellent studies of De Bary (2), the oospores germinate after a rest period either by zoospores or germ tubes and cause the infection of plant tissues. Because of their extremely ephemeral nature, the conidia hardly merit consideration as resting organs, but, nevertheless, they may under certain conditions function as such. If a fungus has two or more annual host plants, it may spread to one or more by conidia after primary infection has resulted from oospores on one; or the fungus may be perennial in one host and spread to another by conidia borne on the former—e. g., *Phytophthora infestans* on the potato and tomato.

The species of Peronosporaceae known to have perennial mycelium are given in Table II.

TABLE II.—Species of Peronosporaceae having perennial mycelium

Name of fungus.	Name of host.	Authority.
<i>Phytophthora infestans</i>	<i>Solanum tuberosum</i> ...	De Bary (1), 1861, Bonn, Germany.
Do.....	do.....	Jensen (6), 1887, Nerilly, France.
Do.....	do.....	Melhus (12), 1913, Houlton, Me.
<i>Phytophthora cactorum</i>	<i>Panax quinquefolium</i> ...	Rosenbaum (14), 1914, Ithaca, N. Y.
<i>Cystopus candidus</i> ...	<i>Capsella bursa pastoris</i> .	Melhus (12), 1913, Madison, Wis.
Do.....	<i>Lepidium virginicum</i> ...	Do.
<i>Plasmopara viticola</i> ...	<i>Vitis vinifera</i>	Istvanffi (5), 1904, Budapest, Austria.
<i>Plasmopara pygmaea</i> ...	<i>Hepatica acutiloba</i> ...	Stewart (15), 1910, Ithaca, N. Y.
<i>Plasmopara halstedii</i> ...	<i>Helianthus diversicatus</i> .	Melhus (12), 1913, Madison, Wis.
<i>Peronospora dipsaci</i> ...	<i>Dipsacus fullonum</i>	Kühn (8), 1875, Halle, Germany.
<i>Peronospora schachtii</i> ...	<i>Beta vulgaris</i>	Kühn (7), 1872, Halle, Germany.
<i>Peronospora alsinearum</i> .	<i>Stellaria media</i>	Magnus (9), 1888, Berlin, Germany.
<i>Peronospora grisea</i>	<i>Veronica hederacfolia</i> ..	Do.
<i>Peronospora effusa</i>	<i>Spinacia oleracea</i>	Do.
Do.....	<i>Atriplex hortensis</i>	Do.
<i>Peronospora ficariae</i> ...	<i>Ranunculus ficaria</i>	De Bary (3), 1876, Bonn, Germany.
Do.....	<i>Ranunculus fascicularis</i> .	Melhus (12), 1913, Madison, Wis.
<i>Peronospora parasitica</i>	<i>Lepidium virginicum</i> ..	Do.
<i>Peronospora viciae</i>	<i>Vicia sepium</i>	Melhus (13), 1915, District of Columbia.
<i>Peronospora rumicis</i> ..	<i>Rumax acetosa</i>	De Bary (3), 1876, Bonn, Germany.

There can be no doubt that the mycelium of several species of Peronosporaceae may become perennial. Of course this can take place only when the host is a winter annual, biennial, or perennial, and quite generally infected. Such plants may live through the winter and renew activity in the spring, when the fungus may sporulate and spread the disease.

The perennial nature of the mycelium of other species of the genus *Phytophthora* has not been studied critically, but there is reason to believe that *Phytophthora infestans* is not the only one that may become perennial. In many cases other species produce oospores prolifically. Butler and Kulkarni (4) believe that on *Colocasias* *Phytophthora colocasiae* may survive the dry seasons of India in the mycelial stage. Another case of perennial mycelium is that of *Phytophthora cactorum* on ginseng (*Panax quinquefolium*), a perennial having a fleshy root, described by Rosenbaum (14). The *Phytophthora* fungus flourishes on the roots, and, according to this author (14), can spread from the roots up the stem to the surface of the soil, and produce conidia which infect the foliage, a case very analogous to *Phytophthora infestans*.

Table II shows that, so far as known, only one species of *Cystopus* has perennial mycelium—that is, *Cystopus candidus* on two hosts, *Lepidium virginicum* and *Capsella bursa pastoris*. Both of these plants may be either annuals or winter annuals, and in both the fungus may become systemic and may survive the winter, provided the host plants live. Unlike *Phytophthora infestans*, *Cystopus candidus* produces oospores pro-

fusely in these two host plants after they mature or are killed by the parasite, but the writer has been unable to find oospores in the young plants during the fall, and this agrees with Magnus's (9) report that oospores are not produced in the seedling plants of spinach infected with *Peronospora effusa* in the fall. Magnus also states that the same is true in the case of *Stellaria media* and *Veronica hederæfolia* infected with *Peronospora alsinearum* and *Peronospora grisea*, respectively.

The number of species of the genus *Peronospora* that may survive the winter in the mycelial stage are more numerous. Table II shows nine. Careful study is in progress in regard to the remaining species of this genus. As also shown in this table, there are three species of *Plasmopara* which may survive the winter in this stage, and this number, the writer is confident, will be increased by further studies.

SUMMARY

(1) There are at least several species of *Peronosporaceae* belonging to four genera that may be perennial in the tissues of their hosts, the mycelium passing the winter either in the aerial or the underground organs of winter annuals, biennials, or perennials.

(2) *Phytophthora infestans* is not an exception in the family to which it belongs as regards perennial mycelium.

(3) The rôle of the mycelium of *Phytophthora infestans* in the tubers of its host is not an unusual one. It may grow from the tubers up the stem to the surface of the soil, sporulate, cause foliage infection, and bring about an epidemic of the disease.

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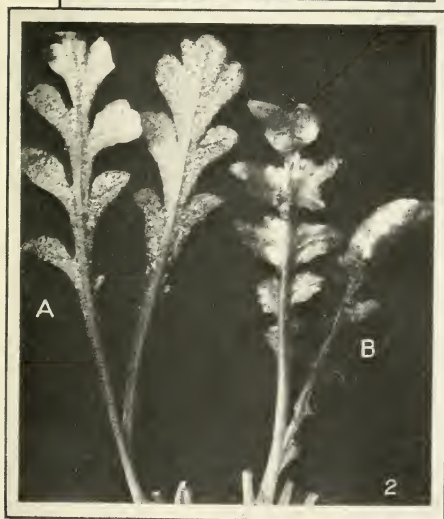
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PLATE III

Fig. 1.—*Cystopus candidus* on *Lepidium virginicum*. This plant was chopped out of the frozen ground on February 22, 1911, and brought into the greenhouse. There days later white pustules of *Cystopus candidus* began to appear on the leaves.

Fig. 2.—A, The two leaves at the left show the amount of sporulation of *Peronospora parasitica* on leaves of *Lepidium virginicum*; B, the two leaves at the right show *Cystopus candidus* fruiting on leaves of *Capsella bursa pastoris*. The pustules developed from mycelium alive in the plants in the winter of 1911.

Fig. 3.—*Peronospora viciae* on *Vicia sepium*. A systematic infection of the downy mildew collected on April 15, 1914, in the District of Columbia. This plant was badly infected when coming through the ground.



HIBERNATION OF PHYTOPHTHORA INFESTANS IN THE IRISH POTATO

By I. E. MELHUS,

*Pathologist, Cotton and Truck Disease Investigations,
Bureau of Plant Industry*

INTRODUCTION

How *Phytophthora infestans* perpetuates itself from year to year has been studied ever since Unger in 1847 (34) ¹ finally proved that the fungus causing the disease is a species of *Peronospora*. No sooner had this fact been established than students began searching for resting organs like those so common in other species of *Peronosporaceae*. As is well known, progress was slow, and the question as to whether *P. infestans* does or does not have oospores ended in a controversy between W. G. Smith (30) and De Bary (4) in the early seventies of the last century. The outcome is too well known to need repetition; suffice it to say that De Bary's negative evidence has been generally accepted.

Recently the oospore question has been taken up anew and bodies resembling oospores have been found by Jones (15, 16, 17), Clinton (9), and Pethybridge and Murphy (27) in pure cultures of the fungus. Although no direct claims that similar bodies exist in the potato plant (*Solanum tuberosum*) have been made, these recent investigations have at least weakened the perennial-mycelium theory, which probably was first advanced by Berkeley in 1846 (5). Like many of the botanists during the first half of the last century, Berkeley unfortunately submitted no experimental evidence to support his contention. The credit of first submitting such evidence belongs to De Bary, who in 1861 in an interesting paper (1) showed that the conidia can not live over winter; that no relation exists between the mycelium of *P. infestans* and of the saprophytes that occur on diseased tubers; that it is impossible to infect potatoes with any of the *Peronosporaceae* that occur on plants common about potato fields; and that the potato fungus is able to spread from diseased seed tubers up into the shoots, sporulate, and renew infection on the foliage.

About 10 years later, Scholtz, Bretschneider, Peters, and Reess took up for the "Central Commission für das Agrikulturchemische Versuchswesen" the problem how *P. infestans* perpetuates itself. They were unable to confirm De Bary (1), and Pringsheim (29), who sum-

¹ Reference is made by number to "Literature cited," p. 100-102.

SPREAD OF THE MYCELIUM INTO THE SPROUTS

When it had been shown that the mycelium was alive in the tuber, at least at some point, its spread into the sprouts was studied. Three boxes (18 by 18 by 6 inches) were filled half full of soil which had never grown a crop of potatoes and which had been steamed for 40 minutes in an autoclave at 15 pounds' pressure. Twelve tubers were partially buried in each box, four of which were sound, the remaining eight being infected with *P. infestans* when harvested. The soil was well moistened with distilled water, and each box covered with a pane of glass. Each box in the series was held at a different temperature—that is, 15° to 20°; 20° to 22°; and 23° to 27° C.

The 8 infected tubers subjected to a temperature of 15° to 20° produced many sprouts, 5 of which became infected during the period under observation. The tubers subjected to 20° to 22° also produced 5 infected sprouts, these appearing during the first 14 days after planting. The greatest number of infections were obtained from the 8 diseased tubers held at 23° to 27°, 13 sprouts becoming infected during the first 14 days after planting. The checks remained free from infection. *P. infestans* seldom sporulated on the parent tuber unless the corky layer was broken, but it was very common on the basal portion of the sprouts growing from infected tubers. In many cases the eyes producing infected sprouts were cut out to learn whether the fungus was present in the tissues immediately surrounding them, and in every case it was found. This showed that the sprout infection was due to the spread of the mycelium and not to spores present in the air, for had the infection been due to spores the checks would have shown as high a percentage of infection as the diseased tubers. Infection by *P. infestans* occurred on sprouts of all sizes, from those barely visible to those nearly 1 inch in length. It was a very common occurrence to find the fungus sporulating first on the lower third of the sprouts, while on the upper two-thirds it was not apparent, but it required only one or two days for the remaining portion to become covered also, which indicates the rate of spread of the mycelium in the sprout tissue.

Naturally discoloration and decay followed the fructification of the fungus. Plate IV, figure 2, shows a potato with diseased and healthy sprouts. This is a late stage of sprout infection, and the tissues of the two infected shoots have blackened. The healthy sprout stands on a portion of the tuber showing no external evidence of the disease, while that part surrounding the diseased sprouts is infected with *P. infestans*. The fungus sporulated only on the sprouts of the diseased tubers, while those arising from the healthy tubers in the same box remained sound throughout, which makes it certain that infection was not by spores present in the air or soil, but by the migration of the mycelium in the tissues of the parent tuber.

This experiment was repeated and has been reported in full in an earlier paper (21). Except in one particular, the results were, in general, alike. In this case a sprout grew out near the surface of the soil from one of the infected tubers. This sprout became infected and the mycelium of *P. infestans* grew out from it into the soil for a distance of about 1 cm. This is not a usual occurrence and happens only when conditions are very favorable for the growth of the fungus. A slight decrease in the moisture content of the soil and the fungus is no longer in evidence, nor does it return if the original moisture condition is restored.

This experiment was again repeated on January 29, but only two sets of temperatures, 15° to 20° and 23° to 27° C., were used. The other set of temperatures was omitted because the supply of tubers was rapidly becoming exhausted, and, besides, it had been shown that temperatures between 15° and 27° were the most favorable. The results were, in general, like those already recorded and need no further consideration. From this series of three experiments, in which infected tubers were partially buried in moist, sterile soil, it is clearly shown that the mycelium of *P. infestans* in infected tubers spreads from the parent tuber into the sprouts, where it may sporulate freely.

Naturally the next step was to learn something as to the behavior of the infected tubers when wholly buried in the soil. To this end 12 sound tubers of the Irish Cobbler variety were artificially infected with a zoospore suspension held in contact with a sprout about one-fourth of an inch long by means of a ring of paraffin, as shown in Plate IV, figure 2. These tubers, together with 6 sound ones as controls, were buried 2 inches deep in a box of wet sterilized soil and placed in a saturated atmosphere at 23° to 27° C. The tubers had gone through the rest period, and in some cases the sprouts were 1 inch long. Eleven days after planting, 4 of the tubers had thrown up shoots. The remaining 8 were dug up to learn their condition, and it was found that in every case the fungus had spread into sprouts other than the one originally infected. Plate IV, figure 2, shows a tuber with the paraffin about the infected eye and the cluster of 5 sprouts at the bud end of the potato. One of the cluster, it should be noted, is free from infection. After the tuber was photographed it was cut and the discoloration typical of *P. infestans* was found at the base of the sprouts. That it was *P. infestans* was further shown by the production of spores and conidiophores on the discolored tissue. The fungus had spread from the initial point of infection over to the point where the cluster of infected sprouts originated from the parent tuber. The four shoots that came through the ground were allowed to remain until April 30, when they were dug up. These were found to be sound, while the parent tubers were totally decayed. The controls remained free from infection by *P. infestans* throughout and developed into normal plants.

In this experiment 4 of the tubers produced healthy plants, while the 8 others were completely overrun before any of the sprouts could reach the surface of the soil. This explains why seed potatoes infected with *P. infestans* give a poor stand. It also shows that the relation of the fungus to the sprouts is the same whether the tubers are wholly or only partially buried. Another significant fact brought out in this experiment was the presence of spores on the surface of the infected sprouts in the soil. This was especially true on sprouts attacked but not wholly killed.

GROWTH OF THE MYCELIUM UP INTO THE SHOOTS

When it became evident that the fungus could grow out into the sprouts from an infected tuber partially or wholly buried in the soil, experiments were outlined to ascertain whether it might not also grow up into the shoots. Thirty tubers were artificially inoculated by introducing living mycelium from pure cultures of *P. infestans* into a wound in each, and all were immediately planted in pots in the greenhouse, the same number of healthy tubers being planted as checks on the same date. None of the plants growing from these tubers showed any signs of infection with *P. infestans*, although they were watched carefully for 71 days, after which the experiment was terminated.

In another experiment 12 naturally infected tubers were planted in pots of steam-sterilized soil. The same number of healthy tubers were planted at the same time as checks. Only 4 of the 12 infected tubers came up, and 3 of these were much less vigorous than the controls. The spindly, sickly looking shoots that grew from the diseased tubers were watched for 47 days, but no sign of *P. infestans* was noted. The tubers were then dug up and found to be wholly decayed, but the stems were sound.

In a later experiment 200 naturally infected tubers were divided into four equal lots and planted directly on the greenhouse bench 1, 2, 3, and 4 inches deep, instead of in pots. An equal number of sound tubers were planted in a like manner as checks. Conditions were made highly favorable for the growth and development of the plants. Seven days after the tubers were planted, a few shoots were noted coming through the ground. The following germination was obtained (Table I).

TABLE I.—Percentage of germination of potato tubers infected with *Phytophthora infestans*

Number of days after planting.	Percentage of germination of seed planted—				Percentage of germination of checks.
	1 inch deep.	2 inches deep.	3 inches deep.	4 inches deep.	
26	33	39	33	13	96
38	39	39	45	44	98

Of the 78 plants that came up 21 were markedly abnormal, while the remaining 57 were quite sound. The sickly plants were covered with bell jars for several days at a time so as to make the moisture conditions more favorable for *P. infestans*, but not a single case of infection either on the basal portions of the stems or on the foliage was found, although the plants were examined daily until the vines died down.

From these experiments and others of a similar nature not mentioned here, it is plain that environmental conditions and the stage of development of the tuber planted determine whether the mycelium may or may not grow up into the shoots. The conditions prevailing in the ordinary greenhouse are not suited to the spread of the mycelium up into the stems.

Believing temperature and moisture to be the chief environmental conditions bearing on the development of *P. infestans*, experiments were made to determine the influence of these factors on the disease.

TEMPERATURE.—The influence of temperature was considered first. Three experiments were made, and, as all were practically the same, a description of one will suffice.

Five 12-inch pots were nearly filled with soil and steam-sterilized. On January 29, 1912, three tubers infected with *P. infestans* were planted 2 inches deep in each of three of these pots, and in the two remaining pots sound potatoes were planted as controls. Two of the pots were placed in a greenhouse where the temperature varied from 15° to 20° C., depending upon the time of the day; the third was placed in another greenhouse where the temperature ranged from 23° to 27° C. With each was placed a pot containing healthy tubers.

The first shoot to appear in the pots kept at 15° to 20° C. came up on February 6, or 8 days after the tubers were planted. The healthy tubers used as controls did not come up as soon as the diseased ones. They were more dormant at the time of planting. It has been observed by several investigators that tubers infected with *P. infestans* germinated sooner than healthy ones. In 12 days all of the diseased tubers had shoots up so high that the panes of glass covering the pots had to be removed. In order to keep the young potato plants in a moist atmosphere, a large bell jar was placed over each of the three pots. Careful examination was made daily. On March 18, or 45 days after planting, the plants were 7 inches tall, but showed no signs of *P. infestans*. At this time the plants held at 15° to 20° C. were dug up to learn the condition of the diseased tubers planted. Three were wholly decayed, while the other three were only half rotten and showed on the remaining portion the typical shrunken areas so characteristic of this fungus. All of the tubers in the control pot were sound. The three tubers partially decayed were now placed in a moist chamber in order to ascertain whether the fungus was still alive in them after being buried 45 days

and after having nourished several plants to partial maturity. Two days later an examination showed that spores and conidiophores were developing on two of the tubers; but no indication of infection was observed on either the leaves or stems which were placed in a moist chamber. Examination on the following day showed no further developments, and, as the potato plants were becoming very much discolored, the observations were discontinued. It should be noted at this point that the fungus was alive and able to sporulate on the diseased tubers after being in the soil for 45 days at a temperature between 15° and 20° C. Had the fungus been latent in the potato leaves and stems, as claimed by Massee (20), it should have developed. The most interesting and important fact brought out in this experiment was the production of healthy vines by a tuber having in it the mycelium of *P. infestans* which remained alive for 45 days.

The two pots which were kept at 23° to 27° C., one containing three infected tubers and the other three healthy tubers, came up a little earlier than those kept at 15° to 20° C. The first shoot came up on February 4, or 6 days after planting, and in 10 days all three of the diseased tubers had shoots up, some of them longer than others. The development of the tubers used as controls was several days behind that of the diseased tubers. Ten days after planting, the shoots were so tall in the pot containing diseased tubers that the pane of glass had to be replaced by a bell jar. The control was treated similarly. Nothing of special interest occurred until March 8, or 39 days after the tubers had been planted, when it was noticed that one of the small shoots growing from one of the diseased tubers appeared water-logged at and a short distance above the surface of the soil. It did not have the normal appearance common to the stems of the other seven shoots in the pot. Upon examination of the water-logged area with a hand lens, a white glistening growth could be seen on the surface. Some of this material was carefully removed and examined microscopically and proved to be spores and conidiophores of *P. infestans*. This infected plant was about 2 inches tall, spindly, light green, and less robust in appearance than some of the other plants in the same pot (Pl. V). The soil was carefully dug away from the stem, and a portion of it below the soil was found to be diseased. This portion gradually became darker as it approached the mother tuber, being brown and doubtless dead at the point of attachment. The parent tuber was nearly all decayed, except one small portion, which was still firm and from which the diseased shoot in question had developed. Free-hand sections made of the portion of the parent tuber where the stem was attached showed the presence of a nonseptate fungous mycelium which was undoubtedly that of *P. infestans*. The tissues of the stem nearest the mother tuber were softer than those higher up, which would indicate that the infection was of longer standing in that section of the stem.

The controls remained free from infection. Because of possible contamination, no further observations were made in the remaining plants in this pot.

This experiment was repeated, beginning February 22, 1912, but instead of large pots six boxes 18 by 18 by 6 inches were employed. Diseased tubers were planted in four of these and sound tubers in the remaining two. Eight were planted in each box, the conditions being exactly the same as in the preceding experiment.

On March 3, or 11 days after planting, one shoot was found just breaking through the soil in one of the two boxes at 23° to 27° C. It seemed perfectly normal both in color and in size, but on examination the next day both the shoot and the surface of the soil immediately surrounding it were covered with a white glistening fungous growth resembling that of *P. infestans*. Upon examining this growth microscopically it was found to be the potato fungus, as suspected. The mycelium on the soil had grown out from the infected shoot and seemed to be confined to the surface of the soil. The soil about the shoot was removed and the underground portion of the stem exposed. It was found to be water-logged just below the surface of the soil and was gradually becoming brownish as the parent tuber was approached. An examination of the parent tuber showed it to be badly decayed at one end, but quite firm at the other. The tissue of the tuber was examined at the base of the young shoot and showed the characteristic blackening due to *P. infestans*. After 48 hours in a moist chamber the fungus fruited profusely. Plate IV, figure 3, shows a cross section of the tuber and the infected shoot.

MOISTURE.—As stated earlier, moisture influences in some way the behavior of the seed tuber and the fungous mycelium contained therein. It was thought worth while to hold infected tubers in comparatively dry rather than very moist soil, as was done in the preceding tests. To this end 24 infected tubers with several living eyes each were planted in steam-sterilized soil on January 13, 1914, in a house where the temperature varied from 15° to 20° C. After 30 days they were covered with a glass-house and kept well watered. Ten of the tubers rotted in the ground before producing any shoots. Thirteen days later a small, spindly shoot growing from one of the tubers showed discoloration just at the surface of the soil. This infection spread upward and the fungus fruited the following day. The remaining 13 were allowed to stand two weeks more, but none of them became infected. When dug up, it was found that all the mother tubers were rotten except two. In these *P. infestans* fruited when the tubers were cut open and laid in a moist chamber, showing plainly that the fungus may remain alive in the parent tubers for at least two months under the conditions of this experiment and also that the mycelium may spread up the stem, even though the infected tuber is not held continuously in wet soil.

In order to test still further the effect of moisture on the growth of the fungus up into shoots, 12 vigorously germinating tubers of the Green Mountain variety were planted in only slightly moist, steam-sterilized sand. These tubers grew rapidly, and in six days some of the sprouts began to break through the surface of the sand. Twelve days later 2 of the 12 tubers were dead. The remaining 10 were potted in steam-sterilized soil and placed in a glasshouse where the soil was well watered and the humidity high. Nine days later one shoot of one of the tubers was badly discolored near the surface of the soil. The discoloration spread up the stem, and after two days the infected area bore conidiophores of *P. infestans* in considerable abundance. When the tuber was dug up, the shoot was found to be diseased throughout its whole length below the surface of the soil. Six days later another tuber showed an infected shoot like the one just described. The remaining 8 mother tubers were dug up two weeks later and found to be entirely decayed. These results tend to show that continuous high moisture content of the soil is not necessary for the growth of the mycelium in the tuber up into the stems. According to the results obtained in these experiments, the soil may be kept comparatively dry until the plants are up. Furthermore, under these conditions the tubers do not rot as rapidly, and a larger number of shoots are produced by each.

INFECTED SEED POTATOES THE CAUSE OF AN EPIDEMIC OF PHYTOPHTHORA INFESTANS

The relation between seed potatoes infected with *P. infestans* and the development of epidemics of the disease under field conditions has received consideration both in Europe and in America, but no one has yet been able to trace and establish beyond doubt the existing relationship. Both De Bary (1, 4) and Jensen (14) claim to have done so, but they made only limited tests in the open in gardens, where conditions are not always comparable to those existing in the field. A large number of field trials having been made with only negative results, coupled with the fact that the mycelium grew up into the stems under laboratory conditions, led the writer to make field trials. For this purpose a section of the country was chosen where this disease occurs annually—namely, northern Maine. Such a section should afford the environmental conditions suitable for the development of all phases of the disease.

FIELD STUDIES IN 1913

The land selected for the experiment had not grown a crop of potatoes for at least five years and had been in hay for the preceding four years. The infected seed planted was selected in the spring from five bins (1,200 bushels each) of potatoes, Irish Cobbler and Green Mountain varieties, grown in the vicinity of Houlton, Me., and held in storage

through the winter. The tubers selected showed various stages of infection; but none were used that did not show at least one living eye (bud). On June 6 the tubers were planted in a 2-acre field of potatoes somewhat isolated from adjoining fields, 256 being planted whole in two rows 8 rods long. In a third row 162 hills were planted with cut infected seed. Alternating with these, three rows were planted with healthy seed, Green Mountain variety, as checks. The seed was planted between 1 and 2 inches deep and the row hilled up so as to cover the sets from 3 to 5 inches. Continuous records were taken of the soil temperature by means of a self-registering Richard soil thermograph. A record was also kept of the rainfall, especially as to the date and approximate amount.

As would naturally be expected, the infected whole tubers sent up shoots more rapidly than the cut seed. Six of the whole tubers had shoots through the ground two weeks after they were planted. On July 6, 30 days after planting, 63 per cent of the whole infected tubers had shoots up; so also did 49 per cent of the cut infected seed and 97 per cent of the tubers planted in the three control rows. After July 6 the percentage increased very little in any of the foregoing cases. On this same date six of the whole diseased tubers that had failed to send up shoots were dug up for examination. Four of these were dead and nearly decayed, while the remaining two had two and five shoots, respectively, which were just ready to break through the surface of the soil. Plate VI, figure 2, shows the condition of one of these shoots immediately after digging. They were taken to the laboratory later and examined for spores of *P. infestans*, but none were found. Subsequently they were placed in a moist chamber overnight, and the next morning small patches of conidiophores bearing spores, which on microscopic examination proved to be those of *P. infestans*, were found scattered over the diseased areas. The infected shoots were very much like those obtained in the laboratory experiments discussed earlier. It should be noted that a few days before the plants were dug up a light shower of rain had fallen, which, it is believed, materially aided the progress of the fungus. These developments in the field experiments are wholly comparable with those in the laboratory, in which the sprouts were attacked and overrun by the disease before reaching the surface of the soil.

On July 13 a very interesting case developed in the row planted with infected cut seed. When the infection was first noted, the discoloration had extended up the stem of the plant only half an inch above the surface of the soil. There was no evidence of spores of *P. infestans*. The weather was clear and the humidity unusually low, a condition not favorable for sporulation of *P. infestans*. The plant was carefully watched the following day, but no evidence of sporulation could be detected. The next

morning, however, the fungus, which, on microscopic examination proved to be *P. infestans*, had fruited, a 500 c. c. beaker having been inverted over the plant in the evening. For three successive mornings after this date evidence of a new crop of spores of this fungus on the little plantlet was found (Pl. VII, figs. 2 and 3). Later the plantlet fell over, owing to destruction of tissue by the fungus and soft-rot organisms which followed. The stem was found to be discolored all the way down to the parent tuber, a distance of 6 inches. The plant was allowed to remain in the field in order to ascertain whether it might infect the foliage of surrounding plants, but no infection developed and the plantlet soon died and dried up. Conditions were probably unfavorable in this case for the development of secondary infections, owing to a poor stand in the row where this infected plant happened to be. This condition makes it necessary for the spores to be carried a greater distance than might have been the case had a higher percentage of the seed planted in this row grown. The stand in the row in question and also the infected hill are shown in Plate VII, figure 3. This case is of special interest in showing that no further development of the fungus occurred, although it did grow up the stem from the diseased parent tuber to the surface of the soil and sporulate.

It was not until July 25 that another case of infection by *P. infestans* was discovered on any of the six rows under experimentation. This case developed in one of the hills growing from a whole diseased tuber. The hill was a vigorous one with 13 shoots, all normal except 3. The smallest of these 3 was 6 inches tall, while the others were fully twice this height. The plantlet was well shaded by the others and was detected only on careful examination of the hill (Pl. VII, fig. 1). When first found on July 25, fully an inch of the stem above the surface of the soil was discolored and a hand-lens examination showed that a fungous growth was present. Some of this growth, collected on a slide and examined microscopically, proved to be spores of *P. infestans*. The weather for five or six days previous to July 25 had been rainy, cool at night, and quite warm in the day time, conditions highly favorable for the rapid growth and spread of the fungus, as demonstrated in the laboratory studies.

The infection spread up the stem into the petioles of the lower leaves and produced spores in abundance. On the 29th, or four days after the infection was first noted, two leaflets in the hill showed infection, and discolored areas appeared on the stems of three of the adjoining shoots about 2 inches above the surface of the soil. The next morning five new leaflets in the hill showed early stages of infection. These infections occurred on leaves in the lower third of the hill, and each day the number of infections increased on the foliage. On July 31 one leaflet was found infected near the top of a plant in one of the adjoining

check rows, and as there was no other evidence of infection in this entire row it seemed quite certain that the spores had come from the hill previously mentioned. On August 5, six days after this stray infection was first noted, 14 others were found immediately below it on the leaflets in the same hill. It seemed quite apparent that the spores had fallen from the infection above and infected the leaves below. The disease continued to spread rapidly until August 10, when a period of hot, dry weather for 10 days checked its development temporarily. At the end of this dry spell, however, it resumed activity, and an epidemic of blight was well under way in this portion of the field. All the plants in the plot, except those on a few short rows of a foreign resistant variety, were killed by late-blight before frost. Four other cases, similar to the one just described, developed between July 25 and August 4. The symptoms in all cases were the same and need not be repeated. In each case the spores produced by the initially diseased shoots infected adjoining foliage and became centers for the spread of the disease.

The plants in the three alternating rows planted with healthy seed were watched for evidence of stem and foliage infection as carefully as those planted with infected seed, as was also the rest of the 2-acre field, but in no case did any infections develop that could not be traced to the centers in the rows planted with infected seed. Of course, after the epidemic was well under way, the source of any single infection was unknown. The significant point and the one on which information was desired was the origin of the very early stages in the development of an epidemic and not the late.

The results of the field tests of 1913 may be briefly summarized as follows: (1) Only 63 per cent of the whole infected tubers and 49 per cent of the cut infected seed grew; (2) the mycelium in infected seed tubers responded the same way in the field as it did in the laboratory experiments; (3) shoots were found that became infected before they reached the surface of the soil; (4) others infected were able to break through the soil and become centers of foliage infection. On these dwarfed infected shoots the fungus fruited and infected the foliage, first in the same hill and later in those adjoining. In this way these hills became the centers for the development of an epidemic.

FIELD STUDIES IN 1914

It is well known that too much reliance can not be placed on the results of 1-year trials under field conditions. This is especially true when dealing with a fungus like *P. infestans*, which is very much influenced by environmental conditions. In view of this fact, it seemed desirable to repeat the field trials of 1913. In 1914, a plot of ground was chosen at Caribou, about 60 miles north of Houlton, Me., where conditions are fully as favorable for the development of late-blight as at

the latter place. A plot of ground was selected that had been lying idle in 1913, but which before had grown several crops of potatoes in succession.

Tubers of the Green Mountain variety showing all stages of infection by *P. infestans* were selected on May 25 from potatoes grown and held in storage throughout the winter in potato cellars at Caribou. Most of them were badly infected, as was natural to expect at this late date. Many had only one living eye, while others, of course, had several. Both whole and cut seed were planted in the same way as already described in the field tests of 1913. In one row 170 whole tubers were planted and 363 in two rows adjoining. On each side of these three rows two rows were planted with sound seed as checks, also of the Green Mountain variety. The planting was made on June 2, when the soil was drier than usual. There was very little rain until July 20, when an inch fell, but, as a whole, the season was drier than that of 1913 and therefore was less favorable for the development of late-blight.

An examination made on July 15 showed that 47.6 per cent of the whole infected tubers, 37.4 per cent of the cut infected seed, and 92 per cent of the healthy seed in the four adjoining rows came up. The low percentage of germination of the infected seed was probably due to two factors, the large amount of infection of the seed with *P. infestans* and the dry weather following planting. The infected seed rotted in the ground in the same way as described in the studies made in 1913.

The first case of infection by this fungus was discovered on July 22, two days after a heavy rain had fallen. It was in a hill grown from a whole infected tuber having nine shoots from 12 to 18 inches tall. Five of the smaller shoots were found to be infected at and below the surface of the soil. The soil was carefully removed from about the hill, and two of the five were found to be discolored all the way from the mother tuber up to the surface of the soil. The three others seemed to have become infected at the surface of the soil, probably by spores borne on the two shoots most generally infected. The infection of neighboring stems in the same hill above the surface of the soil was also noted in the field studies of 1913.

Two days later another hill, also grown from whole seed, was found to be infected. This had 14 shoots, varying from 10 to 18 inches high. The smallest shoot was discolored in the same way as described in the previous case, and upon further investigation the infection was found to extend down to the parent tuber. The fungous infection was evident by the glistening white growth on the stem just above the surface of the soil. None of the older shoots in this hill were infected at this date.

On July 26 one of four shoots in a hill grown from cut seed was found to be infected. These four shoots ranged from 6 to 14 inches in height. Two of the smallest shoots in this hill were infected with *P. infestans*. The

hills in the four check rows were watched as carefully as those in the two rows planted with infected seed, but no infections with *P. infestans* were found.

The development of foliage infection from the three centers described was gradual and wholly comparable to that described in considerable detail in the studies of 1913. It should probably be said in this connection that the first foliage infection was found on July 27, five days after the first case was discovered. By August 14 leaves within a radius of 10 to 20 feet from each center or station were infected with *P. infestans*. A bad epidemic of late-blight was in full swing throughout the whole 2-acre field by September 10. It is plain that the three centers above described formed the starting points for this epidemic. Other centers of infection may have developed subsequently, but no attempt was made to follow the later developments because of the constant recurrence of new foliage infections resulting from the infections about the original centers. The results of the field studies of 1914 confirmed in every way the results obtained in 1913.

The fact that a tuber infected with late-blight may cause an epidemic of the disease raises the question as to the rôle of infected tubers left in the field at harvest time. The majority of these are killed by frost, but a few remain in the soil or get covered during the digging of the crop and may pass through the winter in a living condition. Observations showed plainly that many tubers survived the winter of 1913 in Aroostook County, Me. The fields planted to oats in 1914 that had been in potatoes the previous season were well sprinkled with volunteer potato plants. It is common knowledge among the growers of northern Maine that some seasons volunteer potato plants are very plentiful. Their presence or absence is determined largely by the season, especially by the time and amount of snowfall.

POSSIBILITY OF CONIDIA OF PHYTOPHTHORA INFESTANS BORNE ON THE SEED TUBER REACHING THE SURFACE AND CAUSING FOLIAGE INFECTION

In 1876 De Bary (4) called attention to the possibility of conidia on the seed tuber being able to reach the surface and cause foliage infection. Hecke (12) and Clinton (8) are inclined to believe they function more extensively than the mycelium in the seed tuber. Little is known about the production of conidia on infected potato tissue in the soil or their relation to renewing infection from one year to another. For this reason it was thought advisable to learn something about the possibility of the fungus fruiting on cut seed in the soil and whether the spores functioned.

To this end 31 infected seed pieces were planted in the usual manner on June 22, 1913, at Houlton, Me. The soil was quite dry, and the soil temperature ranged from 10° to 14° C. Three days later they were dug

up for examination, but no spores of *P. infestans* were found. They were again planted and the next day a rain fell, wetting the ground down to the seed potatoes. On June 30, four days after the second planting, the seed pieces were dug up again. Microscopic examination showed that spores and conidiophores of *P. infestans* were present on 26 of the 31 pieces and the growth of the fungus in seven cases was readily visible to the unaided eye. The spores were found to germinate freely in water. These seed pieces were again planted on July 1 and left in the ground for a period of 14 days. At this time careful examination revealed a limited number of spores on 5 of the pieces, but these spores did not appear to be normal; and when placed in water only 3 or 4 germinated. A search was also made for mycelium of *P. infestans* in the soil adhering to the seed pieces, but none was found. The plants that grew from these infected seed pieces were examined daily from the time they came up until the vines were nearly mature, but no infection by *P. infestans* appeared on the foliage.

The above experiment was repeated, beginning on July 2. In this test 14 diseased seed pieces were planted just after a light rain. Four days later they were dug up and examined; on 7 of the tubers spores of *P. infestans* were found. There was no indication that the mycelium was growing saprophytically in the soil adhering to the cut surfaces of the diseased pieces. The pieces were immediately replanted and allowed to grow throughout the season. On July 25 the stem of one of the plants showed infection at the surface of the soil. When dug up, it was found that all of the stem below the surface was diseased and also the parent tuber at the point where the stem originated. This tends to show that the mycelium grew from the parent tuber up into the young shoot and that the infection was not caused by spores in the soil. This plantlet stood in an exposed place and soon died. Spores were produced, however, and a leaf on an adjoining plant became infected. This spread slowly in the leaflet and only a few spores were produced. Finally the leaflet died and dried up and no further infections occurred on any of the plants in the same or adjoining rows. In both these experiments conidia were produced on the seed tuber, but none of them functioned in causing any infections.

In the spring of 1914 further tests were made at Caribou, Me. On June 4, 183 potato seed pieces infected with *P. infestans* were planted in accordance with common practice. The next day it rained. On June 7, 26 of the 183 seed pieces were dug up and examined for conidiophores and spores of the fungus. These were found on 9 of the pieces and the growth was abundant enough to be easily seen with a hand lens. On July 10, 12 more seed pieces were dug up and examined, but no evidence of fructification of *P. infestans* was found. The weather had been clear and warm the five preceding days and the soil was much drier than on June 7. It

may have been that spores were present somewhere on the cut surfaces, but they were not sufficiently abundant to be found even after long and careful search.

On June 20, 20 more of the 183 seed pieces were dug up and examined, but again neither conidiophores nor spores of the fungus could be found. The cut surfaces of the seed pieces in every case had either corked over or started to decay.

No mycelium could be found growing free in the soil about the diseased tubers. No evidence was obtained showing that the fungus continues to sporulate on the seed tubers in the soil. Spores are produced abundantly on the cut surfaces of tubers recently planted in moist soil only, but these disappear in the course of a week or 10 days. In an earlier part of this paper it has been shown that spores may be borne in considerable abundance on sprouts killed before they reach the surface of the soil. Whether these spores ever function in infecting other potato tissue below the surface of the soil has not been shown definitely by any of the earlier workers or by any of the writer's experiments.

There is still another possible source of conidial infection that should be mentioned in this connection. A common practice in northern Maine and other potato-growing sections is to feed the culls to hogs or to dump them in some out-of-the-way place about the farm. In the culls there are usually a considerable number of tubers infected with late-blight. When the skin is ruptured on these, the fungus may fruit. Spores borne in this way may reach potato foliage and lead to infection. Then again, as observed by the writer in numerous cases, tubers infected with late-blight are often dumped in some wet or swampy place on the farm. In two such cases an infection of late-blight was found on the mass of growing plants as early as July 25 and 29. It was impossible to determine how and where the infection originally started, but it was clear that the disease had made a good beginning. It is, of course, needless to say that if such cases developed near a potato field, it might readily become infected.

Whatever may be the possible relation of the conidia to the renewal of epidemics of *P. infestans*, two points are perfectly clear: (1) That spores are borne in the soil on the freshly cut surfaces of infected seed and on sprouts when the soil is sufficiently moist and (2) that the spores probably do not remain viable more than two to three weeks in the soil.

RATE OF SPREAD OF THE MYCELIUM OF PHYTOPHTHORA INFESTANS IN THE POTATO STEM

The rate of spread of infection in the potato stem is of interest because of its direct bearing on the growth of the mycelium from the diseased tuber up through the stem. Healthy plants from 20 to 55 cm. high were exposed to infection with *P. infestans* by spraying a spore suspen-

sion over the plants; and when infections developed on the stems their upper and lower limits were marked with india ink. The infected plants were kept in the greenhouse under conditions favorable for the normal development of the host.

Records were made of infections occurring anywhere on the stem from within 6 cm. of the ground to within a few centimeters of the top. Eight infections within 10 cm. of the ground were kept under observation for four days. The total upward spread of infection in these during the four days was 30 cm., or an average of $3\frac{3}{4}$ cm., and the downward spread was 21 cm., or an average of $2\frac{1}{2}$ cm. Five infections from 10 to 20 cm. above the soil were studied. Two of these were allowed to continue for 48 hours, and the remaining three for only 24 hours. After two days the combined spread up the stem in the five cases was 11 cm., and down, 6 cm., the average spread up and down in each case being $2\frac{1}{5}$ and $1\frac{1}{5}$ cm., respectively. Three stem infections were studied that were more than 20 cm. above the soil; two were between 20 and 30 cm. and one 45 cm. After four days the total spread of infection upward was 23 cm. and downward 11 cm. The average upward growth was $7\frac{2}{3}$ cm. and the downward $3\frac{2}{3}$ cm.

It should be noted that in every case the spread of infection was more rapid up than down the stem and that the fungus progresses more rapidly in young than in old tissues. It is thus evident that it may require only a short time for *P. infestans* to spread sufficiently in the potato stem to reach the surface of the soil, once it is in the basal portion of the shoot. It is likewise quite probable that the fungus grows down the stem from the surface of the soil.

HISTOLOGICAL STUDIES OF THE RELATION OF THE FUNGUS TO THE POTATO STEM

The question arises as to which the mycelium uses when it grows up the infected stem, the cortex, vascular system, or central cylinder. A section of an infected stem always shows that the cortex is discolored, while the rest of the tissues are quite normal. The natural inference from this macroscopic evidence is that the mycelium used the cortex most extensively.

In order to get more exact evidence on this point, infected shoots were killed in various fixatives and were later sectioned and stained. In every case where the cortex was discolored, the cells had collapsed and took the stain very heavily, as shown in figure 1. In such cases the mycelium was not readily seen, and in the majority of cases it was absent. It was sometimes found, however, in the cells between the outer cambium layer and the inner cortical cells, but more often at this stage it was seen growing among the pith cells, as shown in figure 2. Where the cells of the cortex were more normal, or from $\frac{1}{2}$ to 1 cm. above the border line between

healthy and diseased tissue, the hyphæ could be readily seen ramifying between the cells, as shown in figure 3. The mycelium can usually be found higher up in the stem in the cortex than in the pith cells when the disease is growing up the stem from the infected parent tuber. When the cortex has been destroyed it may be found in the pith cells. So far the author has seldom found the mycelium in the vascular system or the wood cells. Histological studies indicate that the mycelium of *P. infestans* spreads up the stem most rapidly in the cortical region when conditions are favorable for its rapid growth.

DEVELOPMENT OF EPI- DEMICS OF PHYTOPH- THORA INFESTANS

One argument used persistently against the theory of resting mycelium being the means of perpetuation of *P. infestans* is the sudden and almost simultaneous outbreak of the disease over wide areas. It has seemed more plausible to many to imagine that some form of resting spore functioned in spreading the disease rapidly each year, as is known to be the case in related species. Massee (20) has questioned the capacity of the conidia of *P.*

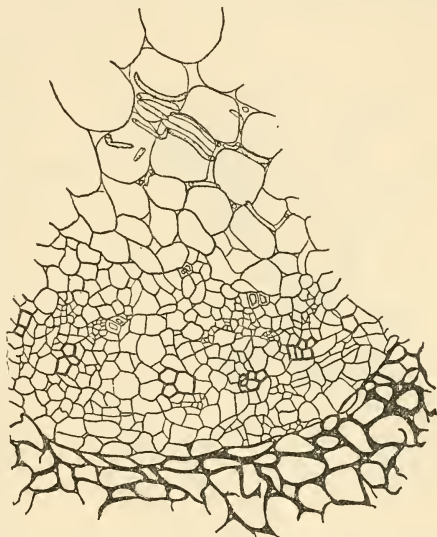


FIG. 1.—Cross section of a potato plant, showing the mycelium of *Phytophthora infestans*, which has killed the cells of the cortex and is a later stage than that shown in figure 3. The mycelium is present among the pith cells. The plant from which this cross section was made became infected like the one in figure 3.

infestans to start an epidemic. He believes that epidemics start from mycelium of the fungus latent in the tissues which becomes active with the advent of favorable weather conditions.

In the fall of 1911 the following experiment was made at Madison, Wis., to learn something as to the development of an epidemic of *P. infestans* under field conditions, with special reference to the rôle played by conidia. It should be mentioned that this fungus seldom occurs in the vicinity of Madison, and, so far as known, it was absent from the State in 1911. The writer is sure it did not occur in the vicinity of Madison that year, and therefore his results were not complicated by its presence. On the even-

ing of August 17, 1911, after a spell of wet weather, two potato plants were sprayed with a suspension of spores of *P. infestans*, the spores having been taken from infected plants in the greenhouse. The inoculation of the two plants was made in the usual way and typical spots became visible in the course of five days. The amount of infection was not extensive. The ground was very moist, owing to the fact that several rains had fallen the previous week, and the weather was continuously cloudy from

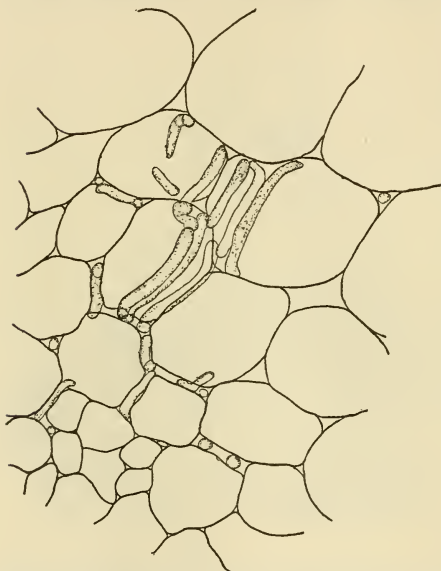


FIG. 2.—A portion of the same section of a potato plant shown in figure 1, showing the mycelium in the pith region of the stem.

August 22, the date infection first appeared, until August 27.

On August 30 infections were found on two plants adjoining those artificially infected, and the next day four more plants immediately adjoining showed infection on several leaves. Careful examination showed no infection on any of the plants farther away. The new infections that had occurred on August 31 were on the six plants immediately surrounding the two artificially infected. The fungus had made no further spread in the half-acre potato plot.

After August 30 new infections were daily found farther and farther from the two plants first infected, and on September 7 infected leaves could be found everywhere throughout the plot, though none of the vines were conspicuously blighted. By this time all the plants within a radius of 8 feet of the two plants initially infected were killed. Farther away the infection was much less in extent, though present in abundance. By September 12 the plot was very badly blighted; not a single plant anywhere was free from infection, and many were wholly dead. No further records except as to the time of harvesting and the amount of loss were kept when the tubers were harvested on October 10. Less than 50 per cent of the crop was fit to put in storage, and less than 10 per cent kept until spring, although held in good storage.

The conclusions to be drawn from this experiment are perfectly obvious. (1) An epidemic can be started by the infection of two plants in a field; (2) two infected plants can spread infection sufficiently to destroy the vines on a half-acre plot in 29 days. That a larger plot, indeed a field of many acres, could be destroyed by one infection is clearly evident.

It might be argued that these conditions were not typical of those occurring under field conditions. On October 14 a visit was made to the potato fields of western New York, where an epidemic was just starting in many of the fields. Infection centers like the one produced by artificial infection in the potato plot at Madison were in evidence in several fields. Another visit to the same fields early in November showed that they had been destroyed by an epidemic of late-blight.

The development of late-blight under field conditions was again followed in the fall of 1913 at Houlton, Me. Careful watch was kept on several fields in that vicinity. The first infection by *P. infestans* was found in the field on August 8, following a few days of wet weather. By going through nine different fields six other centers were found. One typical case will serve to illustrate the prevailing conditions at each center. The infected leaves were always the lower ones of the plant. At

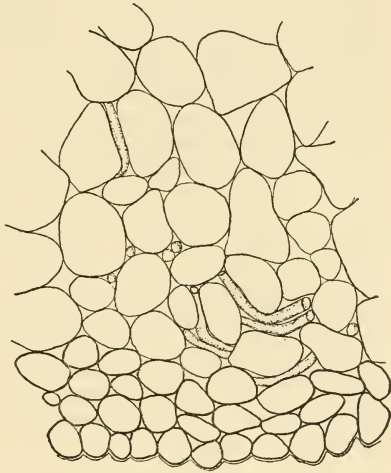


FIG. 3.—A cross section of the cortical region of a potato stem, showing the mycelium of *Phytophthora infestans*. This plant became infected by the mycelium spreading up the stem from the infected parent tuber. This is an early stage of infection, and the tissues of the cortex have not been killed.

the center of the infected area the infections were much more numerous than elsewhere, probably about ten times as numerous. These centers of infection varied from 8 to 40 feet in diameter. If the centers had not become too large, a hill could usually be found that was nearly killed and which suggested strongly that it was the point where the primary infection originated. From August 15 to 28 the weather was hot and dry, and during this period the fungus made little headway. On the date last named a rain fell which facilitated the spread of the disease and caused it to become general though not markedly destructive in the

fields not sprayed with Bordeaux mixture; in other words, epidemics were started from the small areas found early in the season. The spread of the disease was wholly comparable to the above-described developments on the small plot at Madison.

Last summer (1914) three similar infection centers were found in fields near Presque Isle, Me. Such a center is shown in Plate VIII, figure 2. The infected area is set off by a white line. The question naturally arises as to how these centers come into existence. Are they due to the planting of infected seed potatoes or to wind-blown spores? It is impossible to answer either of these queries positively, but in the light of evidence now at hand both are probable. There can be no doubt that seed potatoes infected with *P. infestans* are planted by the growers. This has been observed many times, and in one case 46 seed pieces infected with late-blight were taken from a single barrel of seed potatoes which were about to be planted. None of these were badly infected, but such specimens are more certain to produce infected progeny than those badly diseased, as the latter often rot in the ground.

It may well be, therefore, that these infected centers originate from the infected seed, even although the originally infected shoot is not found. This is probably due to its rapid death after the mycelium reaches the surface of the soil. It soon dries up and leaves little evidence of its presence behind. On the other hand, it is easy to understand how these infected centers might be caused by wind-blown conidia, but it is more difficult to explain their origin without making use of the progeny of infected seed tubers. Although it is not definitely shown how these infected centers originate, in the case of the experimental plots it was clear that they came into existence at the same time that the infected shoots developed. It is also known that seed potatoes infected with *P. infestans* are planted.

RELATION OF THE MYCELIUM IN THE SEED TUBER TO THE PROGENY

Logical as it seems that the shoots and plants produced by diseased tubers should become infected in the same way as the young sprouts, such has not been found to be the case by a large majority of the students of this problem. That the mycelium in the diseased tuber may renew infection from one year to another was first supported by experimental evidence in 1861 by De Bary (1). His evidence, however, was not generally accepted, and in 1876 Pringsheim (29) advanced the alternate-host theory. It should be recalled in this connection that De Bary announced the fundamental rediscovery of heteroecism in *Puccinia graminis* in 1865, which probably influenced Pringsheim (29) and many others in accepting the alternate-host theory as a possibility in *Phytophthora infestans*, where oospores were unknown and infected tubers failed to produce infected plants.

Pringsheim's theory, it must be conceded, won some consideration at the hands of practical growers. This is well illustrated in an early paper by Farlow (11) and an article by Jenkins in 1874 (13). The latter discusses 100 reports made by potato growers on the potato fungus. It is very apparent from these articles that clover or straw was thought by many to be an alternate host for *P. infestans*. This theory, as well as others equally fictitious, was not expounded after 1876, when De Bary published his second paper (4) on this subject. At this time he submitted further evidence supporting the perennial-mycelium theory.

De Bary's theory was not confirmed until about 26 years later, when Jensen (14) repeated De Bary's experiments and obtained infected plants which later became centers of secondary infection. He, like De Bary, worked only in the open, where accidental infection by conidia or by oospores is always possible and where such conditions as moisture and temperature are variable factors. In other words, the technique used by Jensen was no more refined than that used by De Bary 26 years earlier; and he, like De Bary (4), was unable sufficiently to define his method so that his results might be duplicated. In view of this fact it is not surprising that Jensen's researches failed to materially strengthen the perennial-mycelium theory.

During the last 25 years repeated efforts have been made by Boehm (6), Smorawski (32), Hecke (12), Clinton (8), Massee (20), Pethybridge (25), and Jones (17) to grow such diseased plants as were described by De Bary and Jensen from infected seed tubers, both under glass and in the open, but little confirmatory evidence has been obtained. This fact, coupled with the very important discovery by Jones (15), Clinton (9), and Pethybridge and Murphy (27) of resting spores borne by the late-blight fungus in pure cultures, has made the perennial-mycelium theory seem even more questionable. This feeling is liberally expressed by Clinton (8).

The fact that so many students have failed to show the relation of infected seed potatoes to epidemics of the disease may well be due to one or all of three factors: (1) Stage of activity of the tuber, (2) temperature, and (3) moisture of the air and soil.

It is well known that the tuber requires a rest period before it will begin to germinate. If an infected tuber is planted in moist, warm soil before this period has elapsed the tuber rots quickly, owing to the activity of *P. infestans* and soft-rot organisms. If, on the other hand, diseased tubers are held in cold, dry storage until late in the winter or early in the spring and then planted, the tuber makes considerable growth before it is overrun by *P. infestans* and soft-rot organisms. In several of our northern potato-growing sections potatoes are stored at temperatures ranging from 0° to 10° C. until only a short time before planting. The fact that *P. infestans* and soft-rot organisms make little or no growth at

this low temperature explains clearly how infected tubers are able to survive the winter season and are in a condition to make rapid growth when placed in the soil. The statements that tubers infected with *P. infestans* very largely rotted in the ground and that a large majority grew and produced normal plants are both very prevalent in the literature, and the author reports similar experiences in his own experiments. These discrepancies, however, may well have been due to the conditions under which the tubers were stored and their state of germination at planting time. Of course, as will be shown later, the influence of moisture and temperature after planting plays an important rôle.

From infected seed tubers growing rapidly the greatest number of infected sprouts and shoots were obtained in a saturated atmosphere at a relatively high temperature (23° to 27° C.). A temperature of 27° seemed even more favorable than 23° C. This is of interest in view of Vöchting's (35) results to the effect that the optimum for the growth of the potato tuber is about 27° and is not out of harmony with the optimum fixed by Jensen (14) for the growth of the mycelium in the potato tuber. How the fungus spreads in the stem and sprout tissues at temperatures between 23° and 27° C. has been described in an earlier part of this paper. The fungus not only traveled up the stem rapidly but also sporulated profusely at such temperatures. In a paper not yet published it is also shown that the growth of liberated zoospores is more rapid at 23° to 24° C. than at lower temperatures. This is true also where the vines have been inoculated with conidia and zoospores. Although no experiments have been made to establish the optimum for the growth of the mycelium in the diseased tuber, the data cited above show that the mycelium is very active at 23° to 27° C. Whatever may be the optimum for the mycelium in the tuber, this point is clear: That temperatures between 23° and 27° C. are more conducive to the growth of the mycelium than lower temperatures, other conditions being favorable.

Although the state of germination of the tuber and the temperature are important, they do not take precedence over moisture. It need hardly be mentioned that *P. infestans*, by virtue of its phylogeny, is a moisture-loving fungus. To the practical grower it is well known also that an epidemic of late-blight need not be feared in a dry season, while in our northern potato sections a wet season is a sure sign of such an epidemic. The mycelium grows very slowly and absolutely refuses to fruit in a dry atmosphere. It has been shown that the spread of the mycelium is materially retarded when tubers infected with *P. infestans* are buried in dry soil. Again, the necessity of moisture is well illustrated in the case of the isolated plantlet referred to. The fungus made little progress in the stem even after reaching the surface of the soil, and it was only by restoring a moist atmosphere that the fungus fruited. It has also been shown that a greater number of the infected

tubers produced young plantlets when they were allowed to sprout in comparatively dry soil.

De Bary (4) describes a case which is interesting in this connection and serves to emphasize the importance of moisture conditions. A potato plant was found which had become infected by *P. infestans* in the parent tuber. Portions of the stem just above the surface of the soil were infected and discolored, but dry weather prevented the fungus from progressing farther in the tissues or sporulating. This was surely a case where moisture checked the fructification of the fungus. Two similar cases, which are even more striking as showing the close relation of moisture and development of the fungus, are described in this paper. In these the fungus grew up the stem to the surface of the soil and infected the foliage, but the hot, dry weather checked its further spread.

It is not necessary that the optimum conditions for the growth of the fungus should prevail continuously. This is clear from the author's experiments where the tubers were started in dry soil and later transferred to wet soil and the fungus grew up the stem. Too much emphasis can not be placed upon the importance of environmental factors and the state of germination of the tuber in the production of diseased plants from seed infected with *P. infestans*. A combination of these three conditions is not always prevalent in the open nor in the ordinary greenhouse, which may well account for the accumulation of negative data. In this connection may be cited one of several experiments where over 300 tubers were planted in a greenhouse, where the moisture and temperature could not be readily controlled, and not a single infected plant was obtained. Clinton (8), Pethybridge (24, 25), and many others have reported similar results from extensive field trials.

In closing this portion of the discussion it should be pointed out that not all infected tubers give rise to infected shoots and become centers of foliage infection. In fact, only a small proportion function in this way, according to the studies of the author; nor has any method been worked out whereby an infected tuber can be made to give rise to infected plants such as are shown in Plates VI and VII. Whether the progeny of a diseased tuber will or will not become infected is determined by the response of the fungus and host, coupled with environmental conditions. It is known beyond all possibility of doubt, however, that a certain proportion of the diseased tubers planted under field conditions may produce progeny which becomes infected by the mycelium growing up the stem. Once above the surface of the soil, the fungus may sporulate and cause foliage infection on the initial and adjoining hills. Infection spreads rapidly from such an infection center and is the forerunner of an epidemic. Hecke (12) has also noted this early stage in the development of an epidemic. It seems logical to assume that these infection centers start from planted infected seed potatoes.

This method of perpetuation readily explains how *P. infestans* has spread from its native home in South America to every corner of the globe. As pointed out by Jensen (14), it was probably brought to Europe in the mycelial stage in seed potatoes. Likewise, it may well have gone to Australia, New Zealand, North America, and other parts of the world.

MYCELIUM OF PHYTOPHTHORA INFESTANS IN THE SOIL

That the mycelium might live over winter in the soil was possibly first suggested by Kühn (18), who arrived at this assumption because he was unable to grow infected plants from diseased tubers, combined with the fact that the potato fungus occurred year after year. This theory received support later at the hands of Brefeld (7) in connection with his excellent cultural studies of the smuts. He devoted some attention to *P. infestans* also and was probably the first to grow this fungus saprophytically in semipure cultures. It was this significant achievement that led him to support Kühn's theory.

Darnell-Smith (10) has studied the possibility of *P. infestans* living over in the soil. A large number of experiments were made by mincing infected tubers in the soil and planting it to potatoes. He also smeared spores on the tubers when planted, but in no case did he get any infection of *P. infestans*. Some recent experiments by Stewart (33) also bear directly on Brefeld's theory (7, p. 26). He planted healthy tubers in soil mixed with blighted vines and tubers and made conditions highly favorable for the infection of the growing potato plants. No infection of *P. infestans* was obtained.

According to the writer's studies, under certain conditions of moisture and temperature the fungus may grow and sporulate on the surface of the soil to a very limited extent, as described in an earlier part of this paper, but no evidence was obtained showing that it remains alive in the soil for extended periods of time. Jones, Giddings, and Lutman (17) have also recorded the fact that the fungus may spread from infected tissue out over the surface of the soil to a limited extent. Our increased knowledge of culturing parasitic fungi on artificial media, and especially of *P. infestans*, does not permit such deductions at the present time as were made earlier by Brefeld (7).

MASSEE'S LATENT-MYCELIUM THEORY

The early literature on *P. infestans*, then known as the "potato murrain," is full of interesting theories as to its origin. The literature is in every case naturally tinted with spontaneous generation and lack of information as to the life history of the fungus. Fully as interesting is a theory more recently advanced by Massee (20). He maintains that the usual explanation for the sudden appearance of *P. infestans* over wide areas by the dissemination of conidia is inadequate and that the fungus is

latent in apparently healthy potato plants. It is, of course, obvious that Massee makes two radical departures from well-established principles: First, that the rapid dissemination of spores is not sufficient to cause an epidemic; and, second, that mycelium remains latent in the potato tissues.

The development of an epidemic by means of conidia under field conditions has been carefully followed and described in an earlier part of this paper, and the results fully confirm Ward (36) and others. That conidia or asexual spores are able to cause epidemics in the case of a great number of parasitic fungi is well known and needs no further argument. Had Massee demonstrated histologically the presence of latent mycelium in the apparently healthy potato plant as a whole, the latent-mycelium theory would have been worthy of more careful consideration.

WILSON'S SCLEROTIA-LIKE BODIES OF THE POTATO FUNGUS

Another singular theory to account for the perpetuation of *P. infestans* is that proposed by Wilson (37). He believed he had found sclerotia-like bodies on the potato tuber and plant as a whole which were the resting organs of the potato fungus. This theory was later indorsed, strangely enough, by Plowright (28) and W. G. Smith (31). The latter stated that it was his conviction that the bodies Wilson found were of fungous origin, and possibly those figured by Martius (19). These sclerotial bodies were later proved by Murray and Flight (22) to be calcium-oxalate crystals.

Later Wilson (38) reported a more fictitious discovery, that of a mucoplasm existing in the potato plant, which was able to give origin to late-blight.

CONIDIA BORNE IN THE SOIL, RENEWING INFECTION

De Bary early suggested that the fungus might perpetuate itself by means of the conidia, although he considered it very improbable that primary infection often, if ever, takes place in this way. Jensen (14) claims to have found a case where the shoots were killed before they reached the surface of the soil, and the spores on these shoots infected the stem of a healthy plant growing in close proximity. Clinton (8) also cites a case where conidia borne under wet cotton possibly functioned in causing infection in one of his pot cultures. In this paper are recorded further experiments showing that the fungus fruits with great ease on the cut surfaces of the seed tuber and on infected sprouts in the soil, although so far no case has been found where such spores functioned in producing infection above the surface of the soil. It is not impossible, however, that it might happen, and Hecke (12) records such a case.

As stated above, it is not improbable that spores produced on the cut surface of diseased tubers or sprouts may cause infection in some cases, yet the author can not hold with Hecke (12) and Clinton (8) that primary infection due to conidia occurs uniformly throughout a field. In an

earlier part of this paper it is shown how an epidemic developed by artificially inoculating two plants in a plot of potatoes in a section of the country where *P. infestans* did not develop that year and how plants immediately surrounding the two initially infected ones succumbed before any of the others at a greater distance, thereby giving rise to infection centers in the plot in which the vines were killed long before the rest and which increased until it included the whole plot.

Other cases are cited where similar centers known to have originated from the spread of the mycelium up the stem were found and carefully watched under field conditions during the growing seasons of 1913 and 1914. Furthermore, the development of *P. infestans* has been followed for the last three seasons, but no evidence has been obtained to show that it originates uniformly on the lower leaves throughout a whole field. In many cases, when observations are made early enough, the disease is found to originate at some one point and spread outward and radially.

RESTING SPORES OF PHYTOPHTHORA INFESTANS

Resting spores, or oospores, are produced by most of the species of Peronosporaceae. Their function, as is well known, is to bridge the fungus over periods unfavorable for its growth and development. Whether *P. infestans* has oospores has been a bone of contention for the last 60 years. Until recently, however, the prevailing opinion has been that oospores were not produced by this fungus.

During the last decade bodies resembling oospores have been found in pure cultures by Jones (15), Clinton (9), and Pethybridge (26). This discovery has doubtless influenced Pethybridge (25, p. 343) in making the following statement:

It appears to be practically certain that the primary attack of blight each season is due to *spores*, but where these spores come from is not known with certainty, and whether they are similar to those produced on the potato foliage in warm, moist weather in the summer after the primary infection of the crop has taken place, or are of the nature of the thick-walled resting spores produced by species of *Phytophthora* allied to *Phytophthora infestans*, can not definitely be stated at present.

This statement plainly discredits the perennial-mycelium theory and suggests that spores, either conidia or oospores, function in renewing infection. That the mycelium in diseased seed tubers may renew an epidemic of late-blight has been clearly shown in an earlier part of this paper and needs no further argument.

Pethybridge (25) unfortunately does not define the spore that serves to perpetuate *P. infestans*. If he means conidia, there is little evidence to support his contention, as has already been pointed out. On the other hand, it must be conceded that the discovery of bodies resembling oospores in pure cultures of *P. infestans* must be seriously considered when discussing the overwintering of the fungus. At present, unfortunately, there is little positive evidence to support the oospore theory.

It is to be hoped that the recent researches on this problem will afford an angle of approach that will yield positive evidence in the near future.

In closing it should be pointed out that, although *P. infestans* rarely produces oospores in the potato plant, this should not be looked upon as abnormal. As shown in this paper, the production of resting organs is not necessary for the hibernation of the fungus. The mycelium is quite sufficient. There are many species closely related to *P. infestans* that produce few resting spores on certain of their hosts. These may perpetuate themselves from one season to another by means of the living mycelium in the perennial parts of the host plant in much the same way as already described for *P. infestans*. The sparing production of oospores and the hibernation of the mycelium are therefore not uncommon in several species of this family.

SUMMARY

It is clear from the author's experiments that the mycelium of *Phytophthora infestans* spreads in the tissues of the potato tuber and finally reaches the sprouts. The growth of the fungus is retarded when diseased tubers are held in dry soil or at temperatures below 5° C. Infected tubers rot rapidly when placed in warm wet soil. This explains the wide variation in stand obtained by earlier writers. A temperature of 23° to 27° C. and a well-watered soil were found to be the most favorable for the mycelium to spread in the tuber and grow out into the sprouts, both when partially and when wholly covered with soil. Under these conditions the sprouts may become infected from 4 to 20 days after planting, regardless of their size and age. The time required is doubtless influenced by the proximity of the mycelium to the buds and the external conditions.

The mycelium of *P. infestans* may remain alive in seed tubers planted in the soil for at least 45 days, and it is very possible that under conditions less favorable for the soft rots which follow *P. infestans* in the tuber the fungus may live much longer. None of the author's results or observations tend to show that the potato fungus is *latent* in the stems and leaves of plants growing from diseased tubers, as stated by Massee (20).

Laboratory tests showed that the fungus infects not only the sprouts but also the shoots that break through the soil. The mycelium grows from the tuber into the stem, where it travels up to the surface of the soil and sporulates, as held by De Bary (4) and Jensen (14). This usually takes place in the small dwarfed shoots in a hill.

Potato tubers infected with *P. infestans* used for seed purposes and planted under field conditions may cause the development of an epidemic. The mycelium grows from the parent tuber up into the stem exactly as shown in the laboratory experiments. It later sporulates and foliage infection results. Ten such cases were found and followed

in northern Maine during the growing seasons of 1913 and 1914. All except two of these became centers for foliage infection, and severe epidemics of *P. infestans* followed.

Conidia of *P. infestans* may be borne on the cut surfaces and sprouts of tubers when planted under field conditions. As the cut surface corks over or the tuber decays, the fructification of the fungus decreases. Spores taken from tubers two to three weeks after they were first planted showed only limited germinating capacity. No evidence was obtained tending to show that the conidia borne in the soil are instrumental in starting foliage infection.

The mycelium of *P. infestans* spreads most rapidly in the cortical tissues of the stem, where it travels up more rapidly than down.

Epidemics of late-blight may start from a single shoot or hill naturally or artificially infected with *P. infestans*. The infection spreads radially from the initial point of infection during the early stages of the development of an epidemic. These spots of infection in the fields probably come into existence through the planting of seed potatoes infected with *P. infestans*.

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PLATE IV

Phytophthora infestans: Infection of potato tubers

Fig. 1.—Cross section of a tuber which was infected with *P. infestans* and was planted in the greenhouse in rather dry soil. After two months it was dug up and found to be firm and containing living mycelium of the fungus.

Fig. 2.—This tuber was inoculated at the eye surrounded by the paraffin ring. The mycelium ran through the tissues and grew out into four of the sprouts at the bud end of the tuber.

Fig. 3.—Cross section of an infected tuber planted in sterilized soil in the greenhouse which developed a shoot that became infected through the parent tuber.

Fig. 4.—The small stunted shoot which grew from this infected tuber shows the progressive discoloration caused by *P. infestans* growing up the stem.

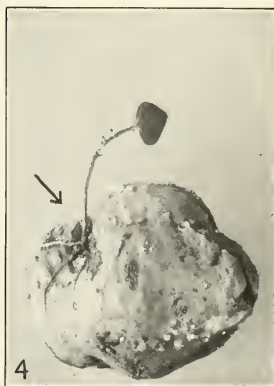
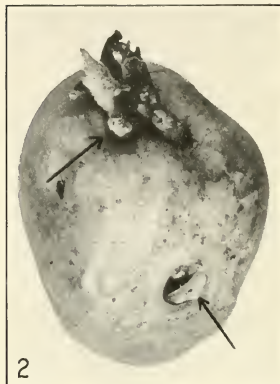
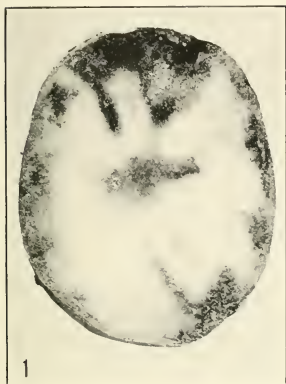




PLATE V

Potato plant showing infection by *Phytophthora infestans*

Three diseased tubers were planted in the greenhouse and held at 23° to 27° C. for 36 days. At this time the small plant in the foreground became infected with *P. infestans*.

PLATE VI

Phytophthora infestans: Infection of potato shoots and plantlets

Fig. 1.—This shoot grew from a diseased tuber planted in the greenhouse under field conditions. Note the discoloration typical of *P. infestans* running up the stem.

Fig. 2.—This shoot, which had not reached the surface of the soil, grew from an infected tuber in the field.

Fig. 3.—This plantlet was the progeny of a diseased tuber planted in the open. It should be compared with the shoot shown in Plate VI, fig. 1, produced in the greenhouse. The same symptoms developed in the field as obtained in the laboratory.

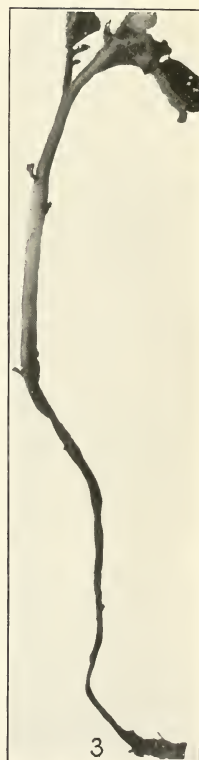




PLATE VII

Phytophthora infestans: Infection of potato plants

Fig. 1.—A hill of potatoes having 13 shoots grown from a whole infected tuber in the field. The smallest shoot, indicated by the arrow, became infected by the mycelium growing up through the stem from the parent tuber.

Fig. 2.—In this hill with two shoots the fungus has reached the surface and killed its host.

Fig. 3.—This shows the hill illustrated in Plate VII, fig. 2, in its position in the row where it grew. Notice the poor stand obtained by planting infected seed potatoes. This hill did not become a center for the spread of *P. infestans*, owing to its isolation in the row and early occurrence.

PLATE VIII

Phytophthora infestans: Infection of potato plots

Fig. 1.—A corner of the plots where infected seed potatoes were planted. An epidemic originated from shoots which became infected through the parent tuber. The four rows of potatoes that still remain standing were of a resistant variety.

Fig. 2.—The area within the white lines shows a spot where infection is much more prevalent than in the surrounding plants. This spot functioned as a center for the development of an epidemic of late-blight in this field.



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ENZYMES OF APPLES AND THEIR RELATION TO THE RIPENING PROCESS

By R. W. THATCHER,

*Chief, Division of Agricultural Chemistry, Department of Agriculture,
University of Minnesota*

INTRODUCTION

Several years ago the writer, at that time connected with the Washington State Agricultural Experiment Station, in cooperation with Mr. N. O. Booth, the horticulturist of that Station, undertook an investigation of the possibilities of slowing up the ripening of fruits by means other than cold storage. While these investigations were in progress, Mr. Booth severed his connection with the Station, but it was understood that he would continue the studies in his new location. For that reason no report of our observations at that time has ever been published; but, since no publication of the results of further work along this line has appeared, the writer feels at liberty to assume that the investigation has been discontinued and to discuss briefly the observations which were made, since they form the starting point for the studies to be reported in this article.

Since the term "ripening" is used to designate various different stages in the development of fruit, it is first necessary to define it as it will be used in this article. Seeds upon ripening usually lose water and go into a resting stage from which germination may take place. But the flesh of an apple (*Malus* spp.) or similar fruit has no definite connection with the life history of the embryo of the seed; hence, its "ripeness" can not be measured in terms of the germination ability of the seed. The fruit itself goes through the following stages of development. There is first a period during which the fruit is growing—i. e., increasing its weight of dry matter. At the end of this period, no matter whether the fruit remains on the tree or is picked off, growth ceases and chemical changes set in which result in the development of the characteristic odor and flavor and later in the disintegration of the flesh of the fruit. When this disintegration proceeds far enough, the fruit becomes soft, mushy, or overripe, and usually at either this or some preceding stage organisms of decay gain entrance to the tissues, and the fruit rots. In the absence

of infection with any germs of disease or decay, the fruit loses water and shrivels up to a withered mass. The group of changes that take place during the second of these stages—i. e., the period between the cessation of growth and the disintegration of the tissue until it becomes soft or mushy—will be termed the “ripening process.”

The object of all storage or preservation of fresh fruit is to slow up the ripening process and so to prolong this period as much as possible. It is a well-known fact that temperature has an important effect upon the rapidity with which these changes take place. It was the object of the studies referred to above to determine whether other factors also influence the rate of these changes and whether they are due in part to infection with disease germs or are wholly enzymic in character.

Two general methods of study were attempted. First, an attempt was made to surround individual apples with a film or coating which would prevent gaseous exchanges and bacterial infection. Repeated efforts to secure a perfect film of this sort with a variety of different materials proved failures; so this method was abandoned. The second method involved the sealing up of the apples in atmospheres of different pure gases under as nearly sterile conditions as possible in order to prevent both disease infection and the ordinary gaseous exchanges. Several large glass bottles, each capable of holding about a peck of apples, were fitted with tight stoppers provided with a glass inlet tube reaching to the bottom of the bottle and an exit tube extending just through the cork. Carefully washed apples were rinsed in a dilute solution of formaldehyde, followed by distilled water, and immediately introduced into the jars and the stoppers sealed in. The apples were of the Alexander variety and were almost ripe—i. e., they would only keep a few days longer without becoming soft. After sealing in the stopper the inlet tube was connected to a supply of pure gas and the latter passed through until no air could be detected in the gas issuing from the exit tube, when the glass tubes were melted off, thus effectively sealing the jars. This method did not, of course, remove the air contained in the tissues of the apples themselves, but this was relatively small in amount.

Each of five jars was filled with one of the following gases: Hydrogen, nitrogen, oxygen, carbon dioxid, and sulphur dioxid; a sixth was sealed with its ordinary air content. No moisture-absorbing material was placed in the jars, as it was thought that this would produce abnormally rapid losses by evaporation from the tissues of the fruit. Further, the recognized chemical changes in the fruit during the ripening process are probably not influenced by the moisture content of the surrounding air, so that the saturation of the air in the jars with water vapor evaporated from the fruit would not be likely to influence the nature of these changes, while constant absorption of this vapor would mean rapid shriveling of the fruit.

The jars were left in a warm, light laboratory and were examined from time to time. The apples in air continued to ripen normally and

in about four weeks were visibly overripe, the lower ones beginning to collapse under the pressure of the weight of the upper layers. Those in oxygen seemed to ripen a little more rapidly, but the difference was not nearly so great as had been expected and was hardly enough to warrant any conclusion that pure oxygen hastened the ripening process. Those which were surrounded by nitrogen and hydrogen did not soften so noticeably, but became discolored and unhealthy in appearance, a phenomenon later observed and reported by Hill (8).¹ After some 8 or 10 weeks, however, these apples also softened into a mushy mass. The apples in carbon dioxid and in sulphur dioxid remained apparently firm and unchanged for a long time, except that the latter gas completely bleached the skins of the apples in its jar, leaving them a uniform creamy white in color. After nearly six months had elapsed, these jars were opened and the fruit examined. That which had been in an atmosphere of sulphur dioxid was firm and solid, but was, of course, so thoroughly impregnated with the disagreeable gas that its quality could not be judged. The apples which had been in carbon dioxid were firm in flesh, possessed the characteristic apple odor, although the gas in the jar had a slight odor of fermented apple juice, and were not noticeably injured in flavor.

It appeared, therefore, that the phenomena ordinarily associated with ripening were greatly inhibited by an atmosphere of carbon dioxid, but that the cause of this inhibition was not wholly a lack of oxygen. It seemed that the changes taking place in the apple were not simple respiratory changes, but probably in large part were internal enzymic activities.

The experiment was repeated the following summer, using raspberries, blackberries, and loganberries instead of apples. It was found that berries which softened in 3 days in air would remain firm for from 7 to 10 days in an atmosphere of carbon dioxid. At this point the studies were interrupted by a change in professional engagements and have not been resumed.

Recently, Hill (8) reported a series of observations so similar in character that interest in the matter was revived; and opportunity being presented for a systematic study of the enzymes of apples by a graduate student² working under the writer's direction, such a study was undertaken, with the results reported below.

CHANGES IN CHEMICAL COMPOSITION OF APPLES DURING RIPENING

The changes in the chemical composition of apples during ripening have been very thoroughly studied by Bigelow, Gore, and Howard (2). The report of their investigations contains a careful review of the literature on the subject, together with significant contributions from the

¹ Reference is made by number to "Literature cited," p. 116.

² The writer's thanks are due to Miss Inez Everett, the graduate student who assisted in the preparation of the material for examination and the carrying out of the several tests.

work of the authors themselves. Briefly summarized, the results of these investigations show that the principal changes which take place in the apple during ripening are as follows:

- (1) A slight but continuous decrease in total acidity calculated as malic acid.
- (2) A gradual decrease in sucrose.
- (3) A gradual increase at first, followed by a later slight decrease, in invert sugar and total carbohydrates calculated as invert sugar.
- (4) The disappearance of starch early in the ripening process.

ENZYMES IN APPLES

The literature which is available to the writer contains very few references to any investigations of the enzymes that are present in apples.

Lindet (9) found in the juice of apples a soluble ferment which causes coloration of the tissues by the absorption of oxygen and the giving off of carbon dioxide, which is inoperative when the juice has been boiled, which may be precipitated from the juice by alcohol, and which oxidizes pyrogallol to purpurogallin. He concluded that the coloration is due to oxidation of tannin by a soluble ferment of the kind designated by Bertrand as laccase (now called "oxidase").

Warcollier (12) is the only other author who reports work on enzymes in apples. Although he was unable to find invertase in apple juice, he believes that it must be present in order to account for the apparent inversion of sucrose during the ripening process. He suggests that the enzyme may have been retained by the apple marc and consequently may have escaped his observation.

The meagerness of the work which has been done along this line is probably due to the fact that the flesh of the apple is not an important element in the physiology of the plant's growth and has little scientific interest to students of plant physiology or biochemistry. But its economic importance and the desirability of knowledge concerning the ripening process as a factor in the storage of perishable fruit products are apparent and, in the writer's opinion, fully justify a thorough study of the subject. The present paper does not constitute an exhaustive report. It does not include, for example, a comparison of enzymic activity of rapidly maturing varieties of apples as contrasted with those which ripen more slowly and, hence, are better keepers. It is believed, however, that the facts here presented will serve as a foundation for such further work as may be found desirable.

EXPERIMENTAL WORK

The apples used in these investigations were secured from an orchardist in the State of Washington and were of varieties known to be good keepers—i. e., slow in ripening in storage. .

PREPARATION OF MATERIAL FOR EXAMINATION

The first problem was naturally that of securing an extract of the cell contents of the apple pulp which would contain the enzymes in active form. Since it was not known whether any or all of these enzymes would be diffusible through the cell walls (extracellular), a preliminary mechanical rupturing of the cells or rendering of them permeable by drying, according to well-known methods of technique in enzyme study, was necessary. Several methods were tried, as follows:

(1) Whole apples were run through a horse-radish grater and the resulting pulp pressed in an ordinary laboratory hand press. The resulting juice was thick, with small particles of pulp, and attempts were made to clarify it by filtration. These were unsuccessful because of the clogging of the filter by the pectin bodies of the juice.

(2) Apples were rasped and pressed as before and the juice allowed to stand for some time, during which the suspended solids settled fairly well, and the supernatant clear juice was decanted. Precautions against enzymic activity during the settling were taken by keeping the settling jars in an ice box.

(3) An attempt was made to secure a dry powder of the apple pulp by drying thin slices in a vacuum desiccator over sulphuric acid; but the large proportion of sugars and pectin bodies in the tissue made this impossible, the slices being gummy and impossible to grind into a powder even after six weeks' exposure in the desiccator.

(4) Thin slices of apple pulp were treated by the acetone-ether method first used by Buchner, Albert, and Rapp (1) in the preparation of *Dauerhefe*, or active dry yeast powder. This process was very satisfactory, the apple slices, after the treatment and exposure to the air overnight, becoming so dry and brittle that they could easily be powdered between the fingers and very easily reduced to a fine powder in a mortar. Several investigators have reported that the enzymic activity of the dry powder so prepared is not less than that of the original tissue, and the writer's observations confirm this. This appears to afford an excellent means of preparation of sugary or gummy materials of this kind for enzyme extractions.

(5) Apples were peeled and cored, and the flesh cut into small blocks. These were then mixed with an equal weight of sharp quartz sand and the mixture rubbed gently in a mortar until uniformly disintegrated. The mixture was then transferred to a fine silk cloth and pressed gently. By this means a limpid juice could be obtained which was nearly free from pectin materials, although slightly cloudy with suspended particles of pulp. Experience has shown that harsh grinding and severe pressure result in diminished activity of the juice, particularly in its oxidase activity, but with gentle manipulation, as above, very active juice can be obtained.

(6) A quantity of concentrated apple juice prepared by Gore (6) by his freezing method was secured and used in some of the tests, since it was thought that this process would be likely to leave the enzymes uninjured in the juice.

EXAMINATION OF DIFFERENT PREPARATIONS FOR ENZYMES

In the earlier examinations reported below, several different preparations were examined simultaneously for the particular type of enzyme which was being sought, in order to avoid any wrong conclusion from improperly prepared material. Experience soon showed, however, that either the acetone-dried powder or the pulp ground with quartz sand would yield active extracts in every case where activity could be found in material prepared by any of the above methods, and one or the other of these two preparations was used in all the later tests. The acetone-dried powder has the advantage that a considerable quantity of material can be prepared at one time for subsequent examination.

DIASTASES

Diastases have been shown by Thatcher and Koch (11) to be readily diffusible into water surrounding cell tissues. It seemed probable, therefore, that if enzymes of this type were present in apple flesh they would appear in juice expressed from pulp after thorough rasping. Samples of clear juice by decantation were secured from three different varieties of apples and tested for diastatic activity. Four separate mixtures were prepared for each variety of juice. The first contained 10 c. c. of a 10 per cent solution of soluble starch prepared by the Lintner method (5), 10 c. c. of the juice in question, and 10 c. c. of distilled water. The second contained 10 c. c. of soluble starch, 10 c. c. of the juice which had been boiled for 10 minutes and made to its original volume with water, and 10 c. c. of distilled water. The third contained 10 c. c. of soluble starch, 10 c. c. of the unboiled juice, sufficient *N/10* sodium hydroxid (NaOH) to exactly neutralize the juice used (determined by a preliminary titration, using phenolphthalein as indicator), and enough distilled water to make the total volume 30 c. c. The fourth, or control, contained 10 c. c. of soluble starch and 20 c. c. of distilled water. The contents of each flask were thoroughly mixed and an aliquot drawn off for the determination of reducing sugars present in the solution. The flasks containing the remainder of the solution were then placed in an incubator for 30 minutes at 40° C., these being the conditions recommended by Sherman, Kendall, and Clarke (10) for all determinations of diastatic activity. At the expiration of this period action was stopped by adding sufficient *N/10* sulphuric acid to make the total volume a *N/200* solution, and an aliquot equal to that taken before the digestion was drawn off for the determination of its reducing sugar content. The soluble proteins were precipitated and the reducing sugars determined by the method out-

lined in the article by Thatcher and Koch (11). The results obtained are given in Table I.

TABLE I.—*Results of tests for diastase in the flesh of apples*

Variety and material.	Reducing sugars.	
	Before action.	After action.
Jonathan:	Gm.	Gm.
Decanted juice.....	0.0192	0.0183
Decanted juice (boiled).....	.0207	.0212
Decanted juice (neutralized).....	.0197	.0192
Control (water only).....	None.	None.
Yellow Newtown Pippin:		
Decanted juice.....	.0113	.0113
Decanted juice (boiled).....	.0217	.0212
Decanted juice (neutralized).....	.0103	.0113
Control.....	None.	None.
Rome Beauty:		
Decanted juice.....	.0103	.0098
Decanted juice (boiled).....	.0207	.0202
Decanted juice (neutralized).....	.0113	.0103
Control.....	None.	None.

At a later date, when other preparations of apple material were available, tests were made of the reducing sugars present in equal aliquots of soluble-starch solution which had been digested for 30 minutes at 40° C., with both boiled and unboiled extracts of these materials, with the results given in Table II.

TABLE II.—*Results of tests for diastases in various preparations made from the flesh of apples*

Material.	Reducing sugars found after action.	
	Active extract.	Boiled extract.
	Gm.	Gm.
Water extract of acetone-dried pulp.....	0.0202	0.0207
Juice concentrated by Gore's process.....	.0356	.0351
Juice from pulp ground with quartz sand.....	.0316	.0316

From these results it is evident that the juice contained no diastases. It appears, therefore, that after the starch disappears from the apples the diastases also disappear. None of the apples which were available for these investigations contained any starch.

INVERTASE

Invertase was tested for in two samples by a method precisely like that used for diastases except that 10 c. c. of a 10 per cent solution of sucrose were used in place of the soluble starch. The results obtained are given in Table III.

TABLE III.—*Results of tests for invertase in the flesh of apples*

Variety and material.	Reducing sugars.	
	Before action.	After action.
Yellow Newtown Pippin:	Gm.	Gm.
Decanted juice.....	0.0113	0.0113
Decanted juice (boiled).....	.0212	.0217
Decanted juice (neutralized).....	.0113	.0103
Control (water only).....	None.	None.
Rome Beauty:		
Decanted juice.....	.0098	.0103
Decanted juice (boiled).....	.0202	.0207
Decanted juice (neutralized).....	.0103	.0113
Control.....	None.	None.

These results being so contrary to what was expected, it was thought best to use material prepared for examination in several other ways in testing for invertase. Accordingly, a water extract was made of some acetone-dried powder from Rome Beauty apples, another sample of the same apples was ground with quartz sand and its juice expressed, and finally a sample of the Gore's concentrated apple juice was diluted to about the same concentration as normal apple juice. Each of these materials was then incubated with sugar solution in the usual way, using unboiled and boiled samples of both the acid and neutralized juice in each extract. The reducing sugars found in the digested mixture from the unboiled or "active" extract and from an equal aliquot of boiled extract are given in Table IV.

TABLE IV.—*Tests for invertase in various preparations from the flesh of apples*

Material.	Reducing sugars found after action.	
	Active extract.	Boiled extract.
	Gm.	Gm.
Water extract of acetone-dried pulp.....	0.0207	0.0207
Juice concentrated by Gore's process.....	.0396	.0396
Juice concentrated by Gore's process (neutralized).....	.0376	Lost.
Juice from pulp ground with quartz sand.....	.0192	.0187
Juice from pulp ground with quartz sand (neutralized)....	.0192	.0192

The results shown in Tables III and IV indicate the absence of any invertase in apple flesh and confirm the observations of Warcollier (12), referred to above. It appears, therefore, that changes during ripening which result in the inversion of sucrose, if they actually occur, must be due to some other cause than the presence of invertase in the apple tissues. The fact that some investigators have not been able to find evidence of this inversion of sucrose during ripening casts some doubt upon its actual

occurrence, there being always the possibility that observed changes in the nature of the sugars present in successive samples may be due to the action of organic acids during the preparation of the samples for analysis.

TANNASE

Determinations of the tannin content of each of the four varieties of apples which were being used by Proctor's modification of Löwenthal's method¹ showed that the flesh of the apples contained the following percentages of tannin: Rome Beauty, 0.208; Arkansas Black, 0.192; Yellow Newtown Pippin, 0.208; King David, 0.132.

It seemed advisable to ascertain, therefore, whether any tannin-hydrolyzing enzyme was present in these tissues. Accordingly, a quantity of pulp from each variety was ground with quartz sand and the juice expressed. One portion of the juice from each variety was boiled and another left unboiled. Aliquots of the boiled and unboiled juice were placed in each of two test tubes, to one of which 2 c. c. of a 10 per cent solution of Merck's tannic acid was added, in order to insure sufficient excess of substrate material. The four sets of four tubes each were placed in an incubator at 40° C. for 24 hours. At the end of this time a few drops of a 10 per cent solution of ferric chlorid were added to each test tube and the intensity of color developed in the tubes containing check boiled and unboiled juices was compared. In no case could the slightest difference in intensity of color be observed, from which it was concluded that the juices contained no tannase.

EMULSIN

Glucoside-splitting enzymes were tested for in boiled and unboiled juices prepared from each of the four varieties of apples by digesting aliquots of these juices with 2 c. c. of a 1 per cent solution of amygdalin for 24 hours at 40° C. In no case was any odor of benzaldehyde perceptible at the end of this time, while check tubes to which emulsin was added gave a pronounced odor after only 10 minutes' contact with the amygdalin used. Hence, it was concluded that the apple flesh contains no enzyme of the emulsin type.

ESTERASES

One of the noticeable changes in an apple during the ripening period is the development of its characteristic odor and flavor, due chiefly to the ester ethyl malonate. Such esters are usually accompanied in nature by a corresponding esterase; hence, it seemed advisable to test the flesh of the apples for an esterase which would hydrolyze ethyl malonate.

Accordingly, apple juice was obtained by the quartz-sand method and a series of test tubes prepared with the following contents: (1) 5 c.c. of apple juice, 5 c. c. of ethyl malonate, and 10 c. c. of distilled water; (2) 5 c. c. of apple juice which had been previously boiled for 10 minutes,

¹ Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p. 1908. See p. 150.

cooled, and made up to its original volume, 5 c. c. of ethyl malonate and 10 c. c. of distilled water; (3) 5 c. c. of apple juice, 5 c. c. of ethyl malonate, sufficient *N/10* sodium hydroxid to render the mixture alkaline in reaction, and enough distilled water to make the total volume the same as in the other tubes; (4), (5), and (6) the same as (1), (2), and (3), respectively, except that a 0.1 per cent solution of steapsin was used in place of the apple juice, as a check upon the reaction conditions. These mixtures were kept in an incubator at 40° C. for 20 hours, after which an aliquot of the mixture was drawn off and titrated with *N/100* sodium hydroxid, using phenolphthalein as indicator, with the results given in Table V.

TABLE V.—*Test for esterases in the flesh of apples*
[Ethyl malonate used as substrate]

Material.	N/100 alkali required.
(1) Apple juice.....	c. c.
(2) Apple juice (boiled).....	9. 2
(3) Apple juice (with excess of <i>N/10</i> alkali).....	7. 5
(4) Steapsin solution.....	^a 39. 8
(5) Steapsin solution (boiled).....	7. 3
(6) Steapsin solution (with excess of <i>N/10</i> alkali).....	None.
	40. 9

^a In addition to *N/10* sodium hydroxid used to make reaction alkaline.

The data presented in this table clearly indicate the presence in the juice of an esterase capable of hydrolyzing ethyl malonate and similar in its action to steapsin. A slight increase of acidity in test tube (1) over that in (2) indicates a slight hydrolytic action even in the acid medium of the unneutralized juice; while in alkaline medium the activity was almost identical with that of the 0.1 per cent steapsin acting in a similar medium.

OXIDASES

Owing to the fact that Lindet's observations (9) mentioned above, the well-known phenomenon of the coloring of apple tissues when exposed to the air, and the qualitative guaiac reaction for oxidases all point to the presence of active oxidases in apples, a quantitative determination of their presence in the different samples available for this investigation was determined upon. Bunzel (3) has shown the objections to the various methods which have been proposed for the quantitative measurement of oxidase activity by various colorimetric determinations and has perfected a manometric method for the purpose. Correspondence with Dr. Bunzel resulted in his kind permission to make use of his apparatus for the investigation of the materials used in this study. Several samples were accordingly taken to his laboratory and their action toward various oxidizable materials determined according to his method. In carrying out the operation, 0.1 gm. of the acetone-dried powder or 2 c. c. of the apple juice obtained by the quartz-sand method were intro-

duced into one arm of the apparatus, 0.01 gm. of the material to be oxidized placed in the other arm, the proper amount of distilled water added in each arm, and the apparatus placed in the constant-temperature box and allowed to stand for 30 minutes to come to a uniform temperature. The apparatus was then closed, the shaking started, and the manometer readings taken at 15-minute intervals. The final readings, with the kind of material and nature of oxidizable reagent used in each case are given in Table VI.

TABLE VI.—*Oxidase activity of various apple preparations toward different oxidizable reagents*

Variety and material.	Oxidizable reagent.	Time of maximum action.	Diminished pressure.
		<i>Min.</i>	<i>Cm.</i>
Rome Beauty:			
Acetone-dried powder.....	Pyrogallol.....	45	0. 10
Do.....	Pyrocatechol.....	60	. 60
Do.....	Guaiacol.....		0
Do.....	Tyrosin.....		0
Yellow Newtown Pippin:			
Acetone-dried powder.....	Pyrogallol.....	45	. 35
Do.....	Pyrocatechol.....	60	1. 75
Do.....	Guaiacol.....	60	. 15
Do.....	Tyrosin.....		0
King David:			
Acetone-dried powder.....	Pyrogallol.....	60	. 20
Do.....	Pyrocatechol.....	60	1. 45
Do.....	Guaiacol.....	60	. 15
Do.....	Tyrosin.....		0
Arkansas Black:			
Acetone-dried powder.....	Pyrogallol.....		0
Do.....	Pyrocatechol.....	45	. 55
Do.....	Guaiacol.....		0
Do.....	Tyrosin.....		0
Juice from pulp with quartz sand.....	Pyrogallol.....	30	1. 45
Do.....	Pyrocatechol.....	30	3. 50
Juice from pulp with quartz sand(boiled).	Pyrogallol.....		0
Do.....	Pyrocatechol.....		0

These results clearly show that apple pulp and apple juice contain an active oxidase, or oxidases, which accelerate the absorption of atmospheric oxygen by pyrocatechol and pyrogallol, and to a slight extent by guaiacol. The activity toward pyrocatechol is much greater than toward the other reagents, indicating the probability that the tannin of apples, which is so readily oxidized on exposure to air under the influence of the oxidases present, is of the pyrocatechol type.

PROTEASES

Protein-splitting enzymes in the flesh of the apple were tested for as follows: A saturated solution of egg albumin was prepared and 5 c. c. of it were placed in each of three test tubes. In one of these, 5 c. c. of apple juice, prepared by grinding the pulp with quartz sand, were added; to the second, 5 c. c. of the same juice, which had been boiled for 10 minutes,

cooled, and made to its original volume; and to the third, 5 c. c. of distilled water. Another set of three test tubes was prepared with the same proportions of materials, but using a 1 per cent solution of Witte's peptone in place of the albumin solution. The tubes so prepared were kept in an incubator at 40° C. for 24 hours. At the end of this time an aliquot of each mixture was drawn off and the quantity of amino acids present in it determined by the ninhydrin method recently proposed by Harding and MacLean (7), using a solution of glutamic acid containing the equivalent of 0.1 mgm. of nitrogen in the amino-acid form per cubic centimeter for the production of the standard color.

The characteristic color due to amino acids appeared in all the tests except the one in which only water and albumin were used. The amino-acid equivalent in each case, as determined by comparison with the standard color, is given in Table VII.

TABLE VII.—*Tests for proteases in the flesh of apples*

Material.	Amino-acid equivalent after action (milligrams of nitrogen).
Unboiled juice + egg albumin	0.12
Boiled juice + egg albumin07
Water + egg albumin	None.
Unboiled juice + peptone10
Boiled juice + peptone10
Water + peptone03

It appears from these data that both the juice itself and the peptone used contained amino acids which would give a blue color with the ninhydrin reagent. But the incubated mixture of unboiled juice and albumin contained more amino acids than that in which an equal volume of boiled juice was used; while with peptone no increase of amino acid was produced by the unboiled juice, and the total amino acid found was just equal to the sum of that found in the quantity of juice and of peptone solution used in the tests. It thus appears that the juice extracted by grinding with quartz sand contains a small amount of some protein-splitting enzym of the trypsin or papain type rather than of the erepsin type. It was concluded, therefore, that the flesh of the apples contains a small amount of protease, to the action of which on the protein material of the apple cells is due the small amount of amino acid found to be present in the juice of the ripening fruit.

PECTINASES

The fact that the flesh of an apple softens and becomes mealy or mushy at the close of the ripening period is generally attributed to the solution of the middle lamella and the consequent separation of the cells of the tissues. The solution of the middle lamella is supposed to be the work of an enzym known as pectinase. It is supposed, therefore, that pecti-

nase occurs in ripening fruits. It was intended at the outset to ascertain whether a pectinase was present in the apples used in this investigation, but review of the literature dealing with methods of detection of pectinase, as summarized by Cooley (4) in a recent article, together with the unsatisfactory results of Cooley's own use of these methods in testing for pectinase in diseased plums, made it appear doubtful that accurate evidence on this point could be secured. Some preliminary tests of the methods which had been suggested confirmed the writer's opinion in this respect, and the attempts were postponed until such time as more satisfactory methods of testing for pectinases have been devised.

ENZYMS IN THE SEEDS OF APPLES

Although the occurrence of different enzymes in the seeds of the apple would not have any bearing upon the ripening processes in the flesh of the apple and, hence, is of no importance to the particular object of this investigation, such an excellent opportunity was offered to test for enzymes in the seeds at the same time that the tests were being applied to the flesh or juice, that it was determined to carry on such a series of tests. Accordingly, a large number of seeds, some 20 gm. in all, were picked out of several apples, and the brown seed coat was picked from each seed. The white seeds were then kept for about two weeks in a vacuum desiccator until they were dry enough so that when crushed they gave off no odor of benzaldehyde, thus indicating that not enough water was present to permit the glucosidase action to occur.

A weighed quantity of the dry seeds was then ground in a mortar with sharp quartz sand until the seeds were thoroughly disintegrated. The material was then preserved in a tightly stoppered weighing bottle until needed for each test. For the tests, 2 gm. of the mixture, equivalent to 1 gm. of dry seeds, were digested at room temperature for 30 minutes with 100 c. c. of distilled water, and a filtered aliquot of this extract was used for the tests. A detailed description of the progress of each particular test is unnecessary in this article, but the results obtained, based upon a comparison of unboiled and boiled extracts with water controls, show the following facts with reference to the presence of the various enzymes which were tested for in apple seeds: Diastases, present in considerable amount; invertase, absent; emulsin, present in considerable amount; lipase, present in small amount; protease, present (hydrolyzes both albumin and peptone); oxidases, absent.

SUMMARY

From the results of these investigations it appears that the only enzymes which participate in the changes in the carbohydrates of apples during the ripening process are oxidases. None of the common types of carbohydrate-splitting enzymes could be found. The fact that the changes which take place during ripening are inhibited by surrounding the fruit in an atmosphere of carbon dioxid, as shown by the experiment described

in the opening paragraphs of this article, is easily explained on the basis of their being oxidase changes, since it is a well-known fact in enzymology that the presence of a large excess of the end products of a reaction generally inhibits the action of the accelerating enzyme in increasing degree as the proportion of the end product increases. Carbon dioxide is undoubtedly the end product of oxidase activity and should therefore accomplish just the result which was found to occur in the jar in which this gas was used.

The small amounts of esterase and of protease which were found in the ripening apples indicate the possibility of the hydrolytic decomposition of the small quantity of essential oil and of protein material contained in the flesh of the apple during the ripening process or subsequent breaking down of the tissue.

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AN AUTOMATIC TRANSPIRATION SCALE OF LARGE CAPACITY FOR USE WITH FREELY EXPOSED PLANTS

By LYMAN J. BRIGGS, *Biophysicist in Charge, Biophysical Investigations*, and H. L. SHANTZ, *Plant Physiologist, Alkali and Drought Resistant Plant Investigations, Bureau of Plant Industry*.

INTRODUCTION

An extended study of the transpiration rate of plants practically necessitates the use of an automatic balance of some type. The present paper contains a review of the various forms of transpiration balances heretofore employed, together with a description of a new automatic transpiration scale of large capacity, so designed that the plants may be freely exposed to the weather. Four of these scales have been in continuous use during the past four summers at Akron, Colo.

Automatic balances may be divided into two classes: (1) The step-by-step type, in which small weights of equal value are added to the scale pan in succession or a counterpoise is advanced in equal steps; (2) the continuous record type, in which the plant is suspended from a spring or from a variable lever or is mounted directly on a float.

RECORDING BALANCES OF THE STEP-BY-STEP TYPE

Vesque (1878)¹ appears to have been the first to employ an automatic balance in measuring transpiration. He made use of the step-by-step principle, a measured quantity of mercury being delivered to a receptacle on the scale pan each time the beam tipped sufficiently to close an electric circuit. His apparatus is illustrated in figure 1, the device for measuring the mercury being shown at *s* and enlarged at B. This measuring device is in principle similar to a large stopcock, in which the plug is only partially bored through from each side so as to form two shallow cavities of equal volume. Either cavity in its upper position becomes filled with mercury from the reservoir *t*. When the circuit is closed, a spring motor is released, which turns the plug through one-half a revolution, delivering the mercury in the cavity to the container *a*, and recording the time of the event by lowering the stylus *p* in contact with the circular plate *v* of the clock H.

Anderson (1894) was the first to employ steel balls of uniform size as weights for a recording balance. The balls were held in a spiral brass tube, with a block at the lower end containing a pocket for one ball. When the balance beam tipped sufficiently to close an electric circuit, the block was moved sidewise and the ball in the pocket dropped into the

¹ Bibliographic citations in parentheses refer to "Literature cited," p. 131-132.

pan of the balance (fig. 2). The weight thus added opened the circuit, and a spring restored the block to its normal position, where the pocket was again filled by a ball from the reserve supply. Anderson did not

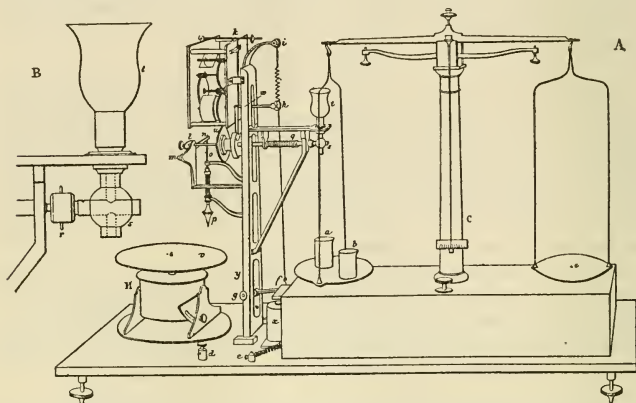


FIG. 1.—Vesque's automatic balance for measuring transpiration. In this apparatus measured quantities of mercury are added to the receiver on the balance pan to counterbalance the transpiration losses.

place the plant directly on the balance, but used his apparatus to register the gain in weight of absorption tubes connected with the transpiration chamber. He does not describe the form of the recording apparatus employed.

Ganong (1905) in his "autographic transpirometer" (fig. 3) combined the ball-dropping and the recording mechanism in a compact and con-

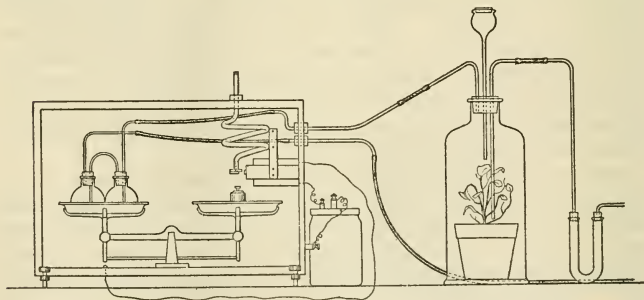


FIG. 2.—Anderson's apparatus for measuring transpiration, in which steel balls are used as weights.

venient form, one electromagnet serving both purposes. Steel balls one-fourth of an inch in diameter were employed as weights. Balls of this size approximate 1 gm. each in weight. The clock was so arranged

that by offsetting the cylinder daily a weekly record could be obtained on one sheet.

Transeau (1911), in working with xerophytes, employed hollow brass balls standardized to 0.4 gm. in place of $\frac{1}{4}$ -inch steel balls of 1 gm. weight, but states that the hollow balls are not as light as could be desired. The writers have found that $\frac{1}{8}$ -inch steel balls weighing 0.13 gm. can be readily used, provided the valve¹ is constructed to fit them.

Woods (1895) used the automatic weighing rain gage of Marvin (1903) as a transpiration balance, the apparatus being modified to record loss instead of gain in weight (fig. 4). In this apparatus the counterpoise is

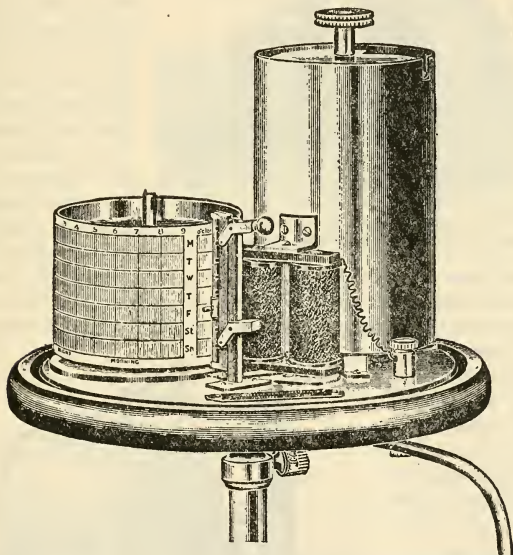


FIG. 3.—Ganong's automatic transpirometer in which steel balls are employed as weights.

moved along the beam in $\frac{1}{10}$ -gm. steps by a screw actuated by an electro-magnet carried on the balance itself. The recorder (fig. 5) is independent of the balance.

Blackman and Paine (1914) have recently described a recording transpirometer operating on the step-by-step principle, in which "water drops are used in place of steel balls, the water being added directly to the soil." Their apparatus has been represented schematically in figure 6. Water is allowed to drip continuously from a Mariotte system. During the greater part of the time the drops are intercepted by a movable

¹ For description of valve, see under "Ball-dropping device," p. 123.

funnel and collected as waste water. When the plant through transpiration causes the balance beam to tip sufficiently to close an electric circuit, the funnel F is withdrawn by the solenoid A, and the water drops fall directly into a receiving tube leading to the soil in the pot. Water is thus

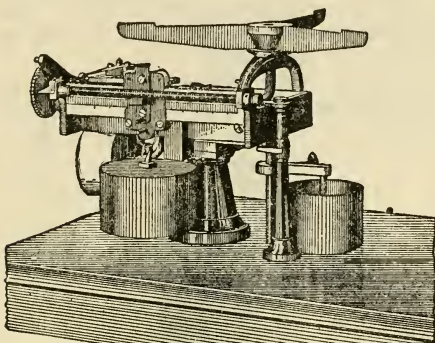


FIG. 4.—Woods' adaptation of Marvin's weighing rain gage as a transpiration balance. In this apparatus the loss through transpiration is counterbalanced by a weight controlled by a screw.

added directly to the pot until the balance tips sufficiently in the opposite direction to close a circuit through a second solenoid B, which restores the funnel to its intercepting position. The time at which the circuit is closed is electrically recorded on a clock drum. The position of the contacts is adjustable, so that the quantity of water

added each time—i. e., the size of the steps—may be modified to suit the transpiration rate. This method is unique and advantageous in maintaining the moisture content of the soil constant throughout the experiment. Under freely exposed conditions, however, the quantity of water added each time would be variable and indeterminate, due to the oscillation of the balance by the wind.

TRANSPIRATION BALANCES OF THE CONTINUOUS-RECORD TYPE

The first continuously recording transpiration apparatus appears to have been devised by Krutizky (1878). It is of interest to note that the first step-by-step recording ap-

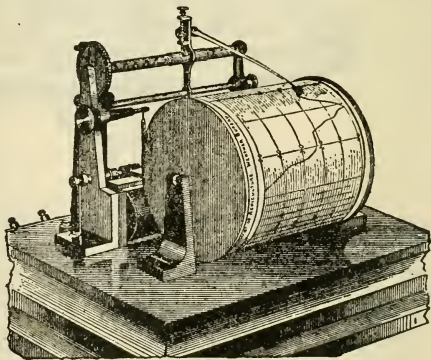


FIG. 5.—The Marvin register used by Woods for recording transpiration.

paratus was described by Vesque in the same year. Krutizky's apparatus is shown in figure 7. The water lost through transpiration from a potometer is continuously replaced through a siphon from a supply con-

tained in a floating cylinder *a*, which rises as the load decreases and traces its movement on the smoked drum of a clock. Like other apparatus involving the principle of flotation, this apparatus is subject to errors arising from changes in buoyancy accompanying changes in temperature.

A transpiration balance devised by Richard Frères (Burgerstein, 1904, p. 8-9) is illustrated in figure 8. The balance is made very insensitive by a heavy bob. The movement of the balance pan from the "down" to the "up" position corresponds to a known

loss in weight, depending on the weight and position of the bob. The movement of the beam is recorded directly on the drum of a clock.

Copeland (1898) has described an apparatus (fig. 9) for recording transpiration in which the weight of the plant is balanced over a pulley by the weight of a partially submerged hydrometer bulb. The pulley shaft rolls on plate-glass supports to reduce the friction. A tracer supported from a second wheel records the motion on smoked paper on a clock cylinder. With its maximum load (3.5 kgm.) the instrument responds to a change in weight of 0.05 gm.

Corbett (1900) has used a large Nicholson hydrometer for measuring transpiration, the plant being placed directly on the pan *a* of the hydrometer *b* (fig. 10). The apparatus is made self-recording by connecting the float with the lever of

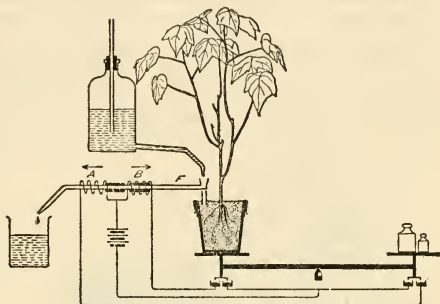


FIG. 6.—Schematic diagram of Blackman and Paine's recording transpirometer, in which water is automatically added to the pot to offset the transpiration loss, so that the moisture content of the soil is kept uniform.

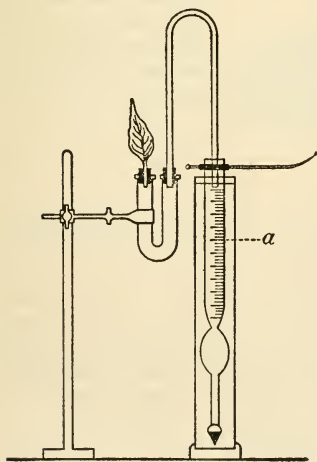


FIG. 7.—Krutizky's potometer for recording transpiration, in which the loss from the potometer is continuously replaced from the supply in the floating cylinder.

an auxanometer. This apparatus, like that of Copeland, is affected by temperature, which changes the density of the water and consequently

its buoyancy. Temperature effects can, however, be practically eliminated by surrounding the hydrometer tank with a water-jacket, through which water is constantly circulating. The sensibility of the apparatus is determined by the cross section of the stem of the hydrometer.

A NEW AUTOMATIC TRANSPIRATION SCALE OF LARGE CAPACITY

The requirements of the transpiration studies at Akron necessitated an automatic weighing apparatus having a carrying capacity of 150 kgm.,

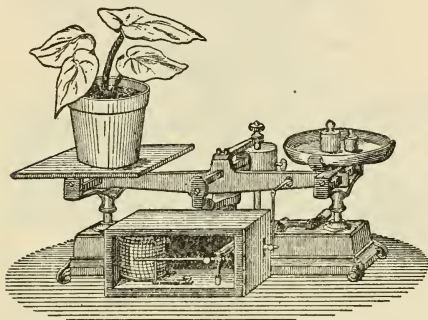


FIG. 8.—The transpiration balance of Richard Frères with its recording apparatus.

capable of operating positively in the wind, and so designed that the plants could be freely and continuously exposed to the weather (Pl. IX). A platform scale with agate bearings having a carrying capacity of 200 kgm. and a sensibility of 5 gm. was chosen for equipment as an automatic balance of the step-by-step type (Pl. X). The scale was fitted with a

short column so as to bring all the mechanism below the level of the top of the pot and was provided with the following auxiliary equipment:

- a. Ball-dropping device.
- b. Ball receiver on beam.
- c. Beam contact and mercury cups.
- d. Oil dashpot on beam.
- e. Spring motor for raising beam.
- f. Adjustable counterpoise for raising the center of gravity of balanced system.
- g. Recorder for registering time at which each ball is dropped.
- h. Batteries and relay.
- i. Case for protecting mechanism from the weather.

The beam of the scale with a part of the auxiliary equipment is shown in fig. 11. The operation of the mechanism is briefly as follows: As the plant decreases in weight, the beam falls until an electric contact is made at U. This closes a relay circuit, with the following results:

1. The ball-dropping device A deposits a ball in the receiver L. The weight of this ball tends to raise the beam.
2. The spring motor, by means of a cam K, raises the beam promptly and positively to its upper position.
3. The time of the event is indicated on the drum of the recorder.

BALL-DROPPING DEVICE.—The ball-dropping device used in our experiments is shown in fig. 12. A commercial telegraph sounder provides an efficient mechanism for actuating the valve. When the circuit is closed, the slide A moves in the direction of the arrow and releases the lowest ball in the tube. The remaining balls are prevented from passing down the tube by the upper septum B, which moves into the tube as the lower septum C moves out. When the circuit is broken, a spring restores the valve mechanism to its original position and the reserve balls slide down the tube so as to rest against the lower septum. The mechanism is now in position to drop another ball as soon as the circuit is again closed.

As the discharged ball leaves the valve it drops into the balanced receptacle D, which tips downward under the weight of the ball, closing the circuit of the recorder through the mercury cups E below. The ball in the meantime rolls into the funnel and is delivered into the ball receiver L, suspended from the balance beam. With this arrangement no record is made unless the ball is actually received in D, and a second ball can not be recorded until the first has been delivered and D has returned to its initial position. In very gusty weather there is occasionally a fluttering of the valve A, two balls being dropped in rapid succession. The second ball simply shoots over D into the waste cup and is not recorded.

The tube holding the reserve supply of balls (fig. 11) is of glass bent into the form of an open spiral, and is joined to the valve tube by a conical adapter. The diameter of the valve tube at the septa must be only slightly greater than the diameter of the balls to insure the valve's working properly, and the tube should taper gradually to this diameter. The distance between the adjacent faces of the two septa should also be equal to the diameter of the ball. Each septum when in its intercepting

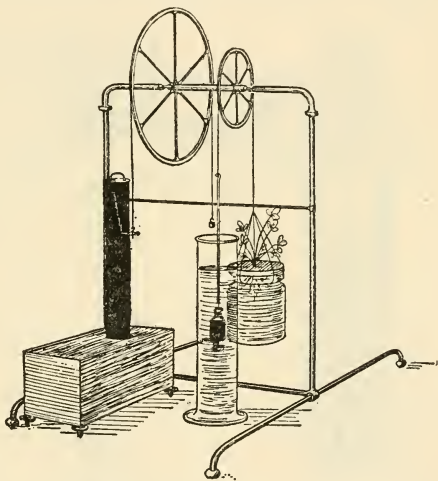


FIG. 9.—Copeland's apparatus for recording transpiration in which the loss in weight through transpiration is counterbalanced by a change in position of a partially submerged float.

The tube holding the reserve supply of balls (fig. 11) is of glass bent into the form of an open spiral, and is joined to the valve tube by a conical adapter. The diameter of the valve tube at the septa must be only slightly greater than the diameter of the balls to insure the valve's working properly, and the tube should taper gradually to this diameter. The distance between the adjacent faces of the two septa should also be equal to the diameter of the ball. Each septum when in its intercepting

position should extend into the tube approximately one-fourth of the tube diameter. It is essential that the valve be accurately made to conform to the particular size of ball used as a weight. The inside of the valve tube should be kept smooth and clean by the occasional use of benzine, and the balls should also be kept polished.

The balls used for weights were three-sixteenths of an inch in diameter and of first-quality hardened steel. They were found to be so nearly uniform in weight that no appreciable error is introduced by assuming them equal. The individual weights in milligrams of 10 balls selected at random were as follows: 437.0, 438.5, 437.2, 437.7, 436.8, 437.6, 437.3,

438.0, 437.5, 437.0. Mean, 437.4. Probable error for a single ball, 0.4 mgm., or 1 part in 1,000.

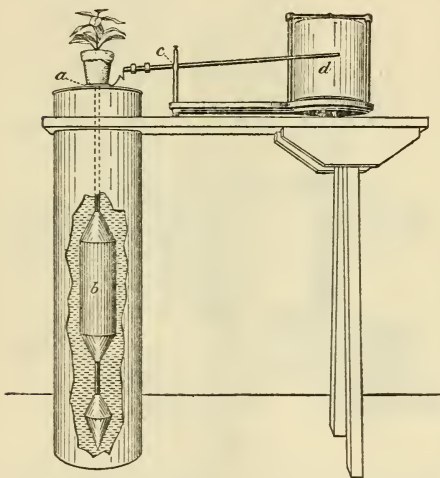


FIG. 10.—Corbett's apparatus for measuring transpiration in which the plant is carried on the pan of a large Nicholson hydrometer.

BALL RECEIVER.—The conical receiver L for the balls is suspended from an extension of the beam (fig. 11) on the same side as the load, since the added weight of the ball compensates for the loss by transpiration. The receiver is suspended from a knife-edge which lies in the plane determined by the two other knife-edges on the beam. The distance

from the central knife-edge is so chosen that the weight of a ball corresponds to a change of 20 gm. in the weight on the scale platform.

The measuring tray shown in Plate XI affords a rapid means of counting the balls dropped during any period without touching them. Each complete row includes 10 balls, and the rows are graduated accordingly on the margin. It is essential that the lower end of the tray be cut obliquely so as to form an angle of 60° with the graduated side.

DASHPOT.—The oil dashpot (fig. 13) is an essential part of the apparatus when the balance is exposed to the wind. The resistance can be adjusted to some extent by turning the perforated plate on the top of the inner cylinder I. The outer cylinder O is mounted directly below the weight support on the beam, to which the inner cylinder is attached by the rod N. (See fig. 11.)

SPRING MOTOR FOR RAISING BEAM.—The dropping of a ball into the receiver is ordinarily sufficient to raise the opposite end of the beam and open the circuit. It sometimes happens, however, when the transpiration rate is high and a gusty wind is blowing, that the beam remains down until the transpiration has been sufficient to require a second ball

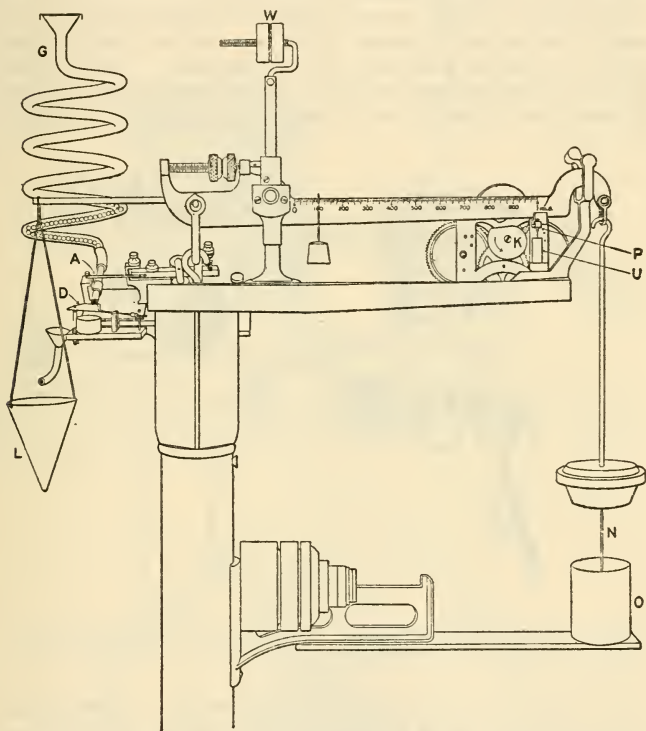


FIG. 11.—View of the beam and auxiliary equipment of the platform transpiration scale designed to carry large pots of plants weighing 150 kgn. or more. As the plant loses weight, the beam falls and the platinum point P closes a circuit through the mercury cup U. This actuates the ball dropper A, which deposits a ball in the receiver L. At the same time the cam K makes one revolution, raising the beam to its upper position and leaving it free to fall. An oil dashpot is provided at O.

to counterbalance the loss in weight. Under such conditions the balance would fail to operate without the intervention of some protective device. This protection is secured by a spring motor which raises the beam to its upper position each time a ball is dropped and then leaves the beam free. The motor, which consists of a strong 8-day clock movement equipped with a fan, F (fig. 14), to reduce the speed, is controlled by

an electromagnet, M (fig. 15). When the beam circuit is closed, the motor is released and raises the beam through a cam, K (fig. 14). When the cam shaft S (fig. 15) has completed one revolution, the arm H on the cam shaft again engages the spring R on the armature T of the magnet, and the motor is stopped.

ADJUSTABLE POISE FOR RAISING CENTER OF GRAVITY OF BEAM.—It is essential that the mercury contact on the beam be closed with a positive motion to avoid the fluttering of the relay armature. This is accomplished by raising the center of gravity until the beam is slightly unstable, by means of an adjustable bob, W, located above the central knife-edge. (See fig. 11.)

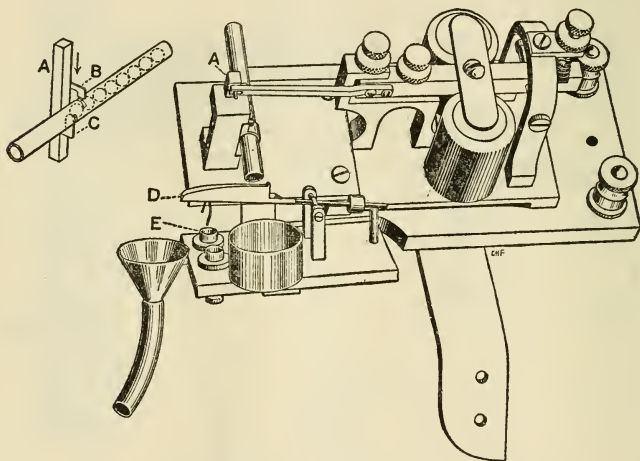


FIG. 12.—Details of the ball-dropping mechanism. The steel ball passes through the valve A into the tipping bucket D, which falls under the weight of the ball and closes an electrical circuit at E to the register.

MARVIN RECORDER.—A convenient type of recorder for registering the time at which each ball is delivered is that devised by Marvin for use in connection with automatic rain gages. This recorder has a drum, 12 inches in circumference, which makes one revolution in six hours and is continuously offset by a screw, so that the four 6-hour periods are recorded side by side on the same sheet. A valuable feature is a zigzag attachment on the magnet, by means of which the tracing pen is permanently displaced each time the magnet circuit is closed. This gives a record which is much easier to read than the ordinary record in which the pen returns to its initial position when the circuit is opened (fig. 16). The dropping of two balls in rapid succession is easily seen in the zigzag

record on account of the double offset, but is difficult to determine in a record of the ordinary type.

PROTECTING CASE.—A tight weatherproof case inclosing the column and beam of the balance protects the automatic equipment from the

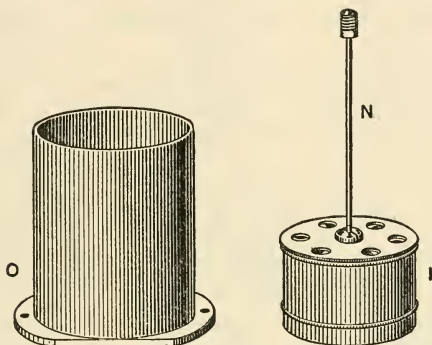


FIG. 13.—Dashpot for preventing the oscillation of the beam during windy weather.

weather. The case is equipped with a removable top and a sliding front. The latter is also supplied with a smaller door through which the apparatus can be observed and adjusted.

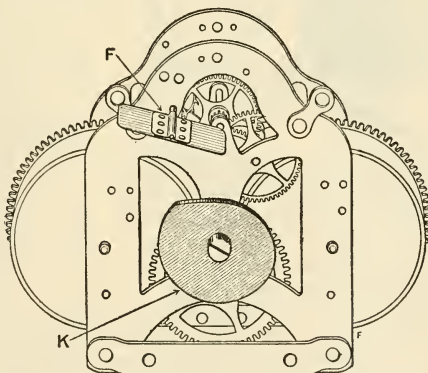


FIG. 14.—Spring motor, showing the cam K for raising the beam, and the fan F for regulating the speed.

ELECTRIC CIRCUITS.—The electrical connections consist of three circuits (fig. 17). A single dry cell, B_1 , operates the relay through the beam contact. The ball valve and the motor release are connected in parallel in a second circuit, B_2 , containing a battery of three or four cells. This

circuit is controlled by the relay contact. The recorder is operated by a third circuit, B_3 , controlled by the tipping bucket on the ball valve. Each circuit is closed only momentarily, and the dry cells usually need to be renewed but once during the summer.

AUTOGRAPHIC RECORDS FROM THE AUTOMATIC TRANSPIRATION SCALE

The results of our transpiration measurements will be presented in other papers, but it seems desirable to reproduce here several daily records illustrating the actual performance of the apparatus. A word of

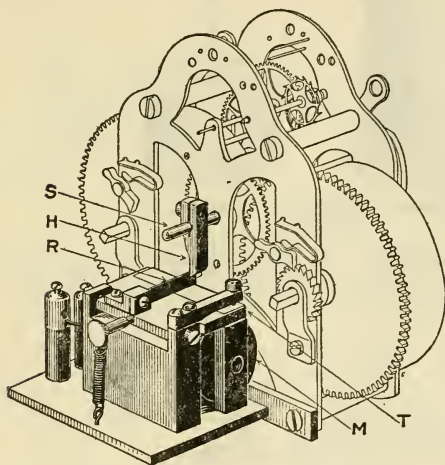


FIG. 15.—Another view of the spring motor, showing the control mechanism. When the magnet *M* is energized, the spring *R* attached to the armature *T* is pulled down, releasing the motor. Raising the beam de-energizes *M*, so that the motor, after making one revolution, is stopped by *H* again coming in contact with *R*.

explanation in connection with the interpretation of the records may be helpful. The clock drum makes four revolutions during the day, so that the record is divided into four 6-hour periods. The pen is offset at the moment each ball is delivered. There are five such offsets or steps in one direction (up, for instance) and then five steps in the opposite direction. Since each offset corresponds to a loss of 20 gm. of water, the interval from the crest to the trough of the graph is the time

required for the transpiration of 100 gm. of water, or from crest to crest, the time interval for 200 gm. loss.

The wheat records shown in figure 16 were taken from a series obtained in 1912 inside the screened inclosure used in the water-requirement experiments. The normal wind velocity was reduced somewhat by the inclosure and by the proximity of other plants. The first record reproduced (July 2, 1912) was obtained on a clear day. It will be noted that the time interval shortens as midday is approached—that is, the transpiration rate increases and attains its maximum value about 3 p. m., after which it falls rapidly. The transpiration at night, represented by

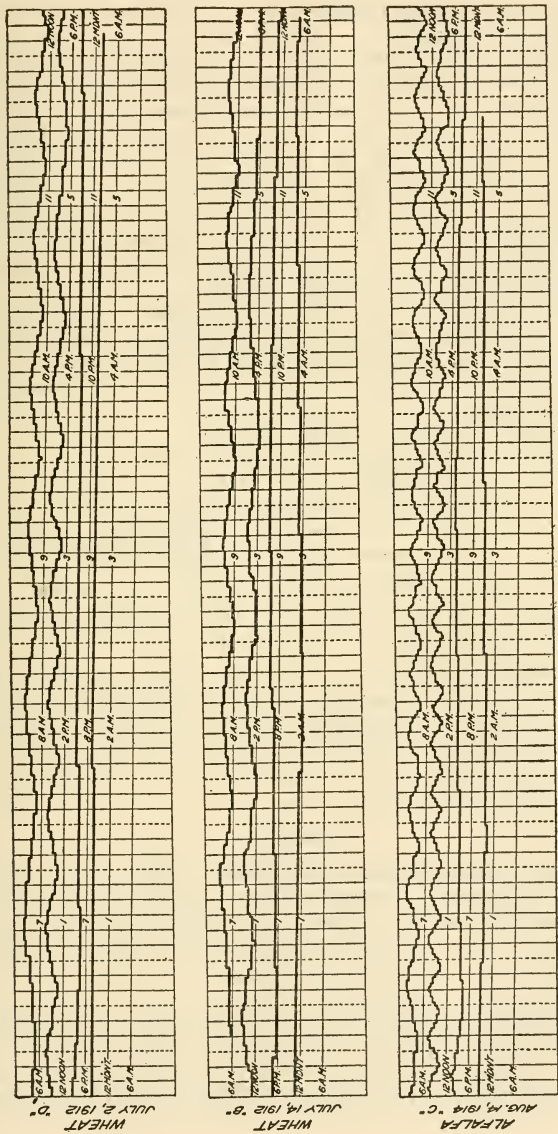


FIG. 16.—Sample records from the automatic transpiration scale. Each step corresponds to a transpiration loss of 20 gm., or 100 gm. from crest to trough of the graph.

the two lower lines of the graph, is seen to be very small as compared with the day transpiration.

The second graph for wheat (July 14, 1912) was selected to show the effect of cloudiness in the afternoon, beginning at 3.30 p. m. The change in the transpiration rate is seen to occur soon after this, and the transpiration between 5 and 6 p. m. is very low compared with that on a clear day, as shown by the first chart. The transpiration during the night of July 14 was higher than during the night of July 2. Automatic measurements with a wet-bulb instrument show that the air contained less moisture during the night of July 14 than during the night of July 2, which would account for the increased transpiration. The temperature on the two days was practically the same.

The third chart shows a record of a pot of alfalfa, taken outside the inclosure. The plants were freely exposed to the wind, which ranged in velocity from 7 to 14 miles per hour during the morning and from 2 to 5 miles during the afternoon. Over 8 liters of water were transpired

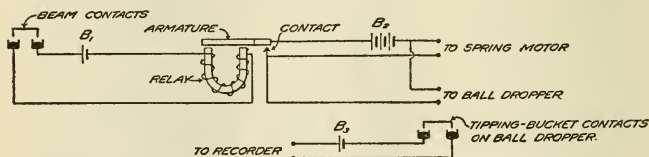


FIG. 17.—Wiring diagram of the electric circuits of the automatic transpiration scale.

during the day, and it is of interest to note how closely this loss is confined to the daylight hours.

The transpiration recorded on the three record sheets reproduced in figure 16 is plotted in rectangular coordinates in figure 18, showing for each pot of plants the transpiration rate in grams per hour for each hour of the day. It may be added that the pots used were equipped with sealed covers, so that the loss of water by direct evaporation from the soil was practically eliminated.

SUMMARY

This paper describes an automatic transpiration scale of 200 kgm. capacity and 5 gm. sensibility, designed for use in connection with the large culture pots employed by the writers in water-requirement measurements. The apparatus is so constructed that the plants may be freely exposed to wind and weather. Steel balls are used as weights, as in Anderson's balance, each ball corresponding to a change in weight of 20 gm. A spring motor is provided to lift the beam positively when a ball is dropped, which is an essential feature when plants are exposed to wind. The apparatus works very satisfactorily except in the presence of whirlwinds or sudden gusts, which lift the plants and tend to give a transpiration

rate which is momentarily too high. Special provision is made to prevent two balls being delivered to the beam in rapid succession, and no record is made unless a ball is actually delivered to the ball container on the beam. Four of these automatic scales have been in use during the past four summers at Akron, Colo., and continuous records have been secured during these periods. The results of these measurements will be discussed in other papers.

A brief review is also given of other forms of transpiration balances, which are divided into two classes: Those operating on the step-by-step principle, which includes the balances here described, and those of the continuous-record type. The first class includes balances in which the adjustment is secured by adding small weights (solid or liquid) of equal mass or by moving a counterpoise in uniform steps. In the second class the plant is suspended from a spring, or from a variable lever, or is mounted (directly or indirectly) on a float.

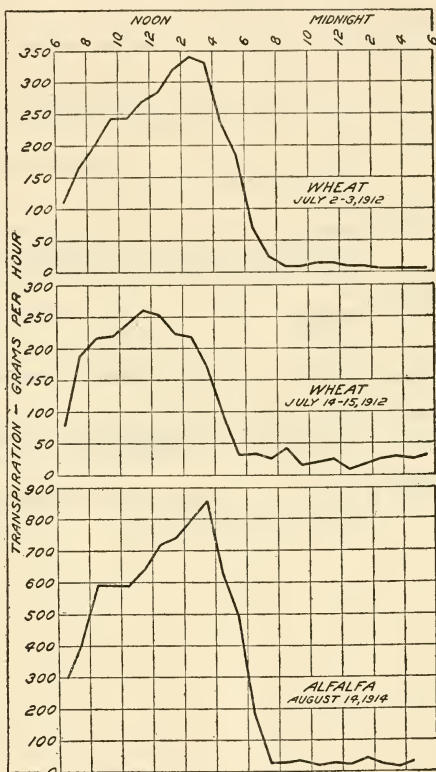


FIG. 18.—Transpiration graphs corresponding to the three records given in figure 16, plotted in rectangular coordinates.

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PLATE IX

Fig. 1.—Four automatic balances in operation at Akron, Colo., June 19, 1912, with the front of the box containing the mechanism open. The recording device is shown just beyond the first box. A separate recorder is used for each instrument.

Fig. 2.—Automatic balances, Akron, Colo., July 24, 1912; boxes closed and recorders covered. Except when being adjusted, this is the condition in which the apparatus is maintained.



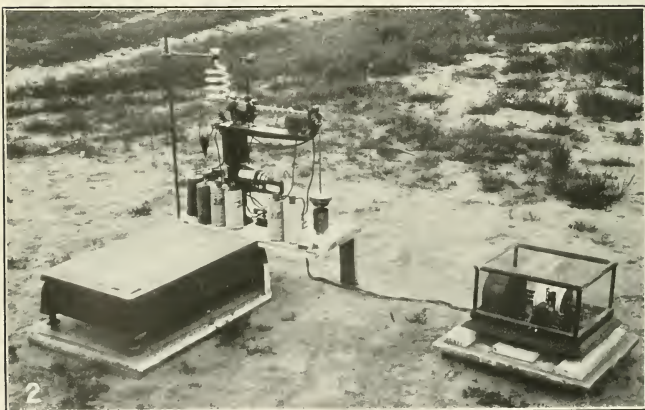
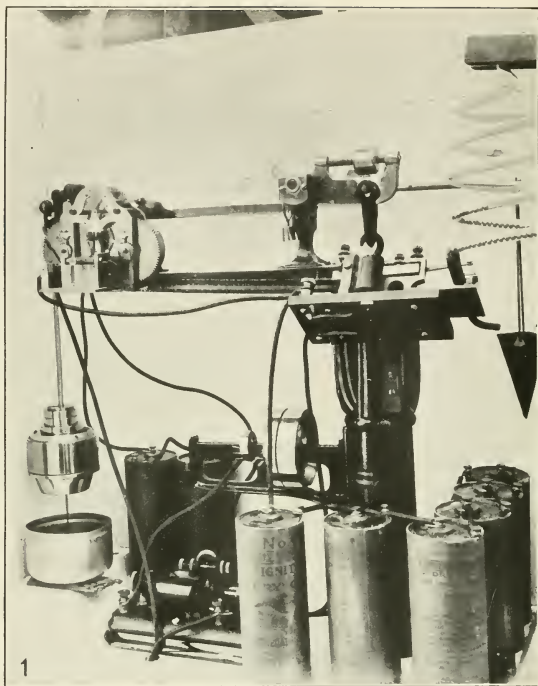


PLATE X

Fig. 1.—Front of balance, cover removed, showing mechanism. The spiral glass ball container will be seen in the upper right-hand corner, the balls passing down through the ball dropper into the basket shown at the extreme right. The spring motor for raising the beam is shown at the upper left-hand side. The dashpot is seen below the weight carrier.

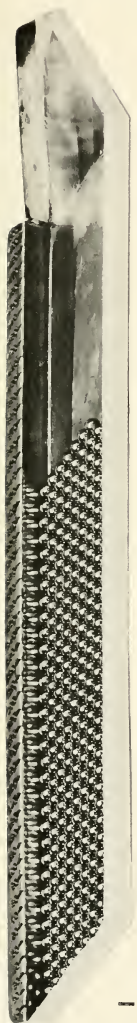
Fig. 2.—General view of automatic balance with case removed.

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PLATE XI

Fig. 1.—Measuring tray used in counting total number of balls delivered to the container on the balance arm during the 24-hour period.

Fig. 2.—Another view of the measuring tray looking vertically downward on the tray, showing the 60° angle which the base makes with the graduated side. This tray contains 255 balls, as will be seen by reference to the graduations.



1



2

PARASITISM OF COMANDRA UMBELLATA

By GEORGE GRANT HEDGCOCK,

Pathologist, Investigations in Forest Pathology,
Bureau of Plant Industry

One of the most important and most injurious of the stem or blister rusts occurring on pines is *Peridermium pyriforme* Peck, which attacks *Pinus (murrayana) contorta* Loud., *P. ponderosa* Laws., and *P. ponderosa scopulorum* Engelm. in the western United States, *P. divaricata* Du Mont de Cours. in the Northern States, and *P. pungens* Michx. and *P. rigida* Mill. in the Northwestern States. *Peridermium pyriforme* is a heteroecious rust and is dependent for its existence upon its alternate, or summer, stage, which occurs on species of *Comandra*.

The problem of the eradication of this important rust being so intimately associated with plants of *Comandra* spp. led the writer to investigate their manner of growth and means of propagation. It was found that the plants of at least two species, *C. pallida* A. DC. and *C. umbellata* (L.) Nutt., have apparently become largely dependent on parasitism for their continued existence. The other two North American species, *C. livida* Richards, and *C. richardsiana* Fernald, resemble the former species in appearance and habit and are probably equally parasitic in their nature.

The writer has carefully examined the root system of living plants of both *C. umbellata* and *C. pallida*, but only of dried specimens of the other two species. The former have long underground rootstocks which bear here and there small roots or rootlets usually less than 5 inches in length. These rootlets branch sparsely and are nearly always attached to the roots or underground stems of other species of plants. At the point of attachment there is formed by the root of *Comandra* spp. a nearly hemispherical disk or holdfast. This holdfast is either superficial or slightly embedded in the cambium layer of tissues of the host, but does not send out haustoria, as is the case in species of *Razoumofskyia* on the limbs and trunks of coniferous trees. The chief function of the roots of *Comandra* spp. appears to be that of attachment to host plants for the purpose of obtaining nourishment and a water supply. Plants of *Comandra* spp. frequent dry, rocky soils, which often have a low water content.

Plants of all these species of *Comandra* bear leaves; and although attached as parasites to the roots of other plants, they are not entirely dependent upon their host plants for organic compounds, since they are able to further elaborate these compounds in the liquids received from

their hosts. In this respect their development is similar to that of plants of species of *Phoradendron*.

Both *C. umbellata* and *C. pallida* very commonly are associated with and parasitic upon species of *Vaccinium*, but are not at all dependent upon this genus for host plants. This has especially been noted in the case of *C. pallida* in the States of Colorado, Montana, Nebraska, South Dakota, and Wyoming, and in *C. umbellata* in the States of Connecticut, Maryland, Michigan, Minnesota, New Jersey, New York, Pennsylvania, Vermont, Virginia, and Wisconsin, and the District of Columbia. Plants of both species are parasitic upon a great variety of plants belonging to widely different sections of the Spermatophyta. No attachment to plants of any member of the Pteridophyta has been noted.

C. umbellata has been found by the writer as a parasite on the roots of the following species of plants in the Eastern States:

<i>Acer rubrum</i> L.	<i>Meibomia paniculata</i> (L.) Kuntze.
<i>Achillea millefolium</i> L.	<i>Panicum</i> sp.
<i>Andropogon virginicus</i> L.	<i>Poa compressa</i> L.
<i>Angelica villosa</i> (Walt.) B. S. P.	<i>Poa pratensis</i> L.
<i>Antennaria plantaginifolia</i> (L.) Richards.	<i>Populus tremuloides</i> Michx.
<i>Aster ericoides</i> L.	<i>Potentilla monspeliensis</i> L.
<i>Aster macrophyllus</i> L.	<i>Quercus coccinea</i> Muenchh.
<i>Aster patens</i> Ait.	<i>Quercus digitata</i> (Marsh.) Sudw.
<i>Aster undulatus</i> L.	<i>Quercus marilandica</i> Muenchh.
<i>Baptisia tinctoria</i> (L.) Br.	<i>Quercus nana</i> (Wood) Britton.
<i>Betula nigra</i> L.	<i>Rhus copallina</i> L.
<i>Betula populifolia</i> Marsh.	<i>Rosa blanda</i> Ait.
<i>Carex</i> sp.	<i>Rosa canina</i> L.
<i>Castanea dentata</i> (Marsh.) Borkh.	<i>Rubus canadensis</i> L.
<i>Chimaphila umbellata</i> (L.) Nutt.	<i>Rubus procumbens</i> Muhl.
<i>Chrysopsis mariana</i> (L.) Nutt.	<i>Rubus villosus</i> Ait.
<i>Comptonia peregrina</i> (L.) Coulter.	<i>Solidago bicolor</i> L.
<i>Danthonia compressa</i> Austin.	<i>Solidago caesia</i> L.
<i>Fragaria americana</i> (Porter) Britton.	<i>Solidago juncea</i> Ait.
<i>Fragaria virginiana</i> Duchesne.	<i>Solidago nemoralis</i> Ait.
<i>Gaylussacia frondosa</i> (L.) T. and G.	<i>Solidago speciosa</i> Nutt.
<i>Hieracium venosum</i> L.	<i>Spiraea salicifolia</i> L.
<i>Ionactis linariifolius</i> (L.) Greene.	<i>Vaccinium atrococcum</i> (A. Gray) Heller.
<i>Lespedeza violacea</i> (L.) Pers.	<i>Vaccinium nigrum</i> (Wood) Britton.
<i>Lysimachia quadrifolia</i> L.	<i>Vaccinium vacillans</i> Kahn.

In addition to the foregoing and incomplete list there must be added at least three unidentified species of grasses.

During the last three years a number of attempts, with varying success, have been made at Washington, D. C., to grow plants of *C. umbellata* and *C. pallida*, both by germinating the seed and by transplanting rootstocks to beds and pots in greenhouses. In every case where living rootstocks unattached to host plants have been transplanted to pots or

beds without the host plants present, little or no growth on the part of the plants of *Comandra* spp. has taken place, and the plants eventually died. Successful results in growing these species have been accomplished by only two methods: First, by transplanting sods containing the plants of *Comandra* spp. from out of doors to the greenhouse without breaking the attachments of the roots of the parasite to those of the host; second, by planting seed in flats in the fall out of doors and germinating them in the presence of the roots of host plants after exposing the seeds to freezing temperatures by allowing the flats to remain out of doors all winter.

Dr. E. P. Meinecke, of the Office of Forest Pathology, reports by letter that he has three plants of *C. umbellata* raised from seed sown in 1913, which remained dormant till 1915, when they germinated and grew without any host plant. These plants were 5 inches high on July 17, 1915. This is positive proof that this species of *Comandra* can live without parasitism if necessary. It remains to be seen whether these plants will continue to grow indefinitely without the presence of host plants.

The results from our experiments indicate that when the rootstocks of plants of *Comandra* spp. are broken entirely loose from their root attachment to host plants they usually die through an inability to re-attach themselves. These new data on a subject which apparently has not been previously investigated indicate a greater degree of parasitism in species of *Comandra* than has hitherto been suspected, and will render more obvious the desirability of the destruction of plants of *Comandra* spp. in the vicinity of forest-tree nurseries.

SEPARATION OF SOIL PROTOZOA¹

By NICHOLAS KOPELOFF, H. CLAY LINT, and DAVID A. COLEMAN,

Research Fellows, The New Jersey College for the Benefit of Agriculture and Mechanic Arts

Some interesting problems have been suggested by the contention of Russell and Hutchinson (9, 10)² that protozoa are one of the limiting factors in soil fertility, because they feed upon and consequently limit the numbers of soil bacteria. Before the agricultural scientist can successfully formulate a complete explanation of the phenomena concerned with the function of protozoa in soils it is essential to establish certain fundamentals in methodology. Russell and Hutchinson (9, 10) and Cunningham (2, 3) have presented some valuable information concerning the depression of bacterial numbers as a result of inoculation with cultures of protozoa. The writers entered upon an investigation of a similar nature, with an attempt to base their work upon the use of protozoa-free cultures of bacteria, and bacteria-free cultures of protozoa.

But little mention is to be found in the literature regarding the separation of the different kinds of protozoa from each other and from bacteria. Russell and Hutchinson (9, 10) and Fred (4) have employed an efficient method of filtration for obtaining cultures of protozoa, but they do not offer any further experimental data concerning such separations. Cunningham (2, 3) has made use of a single-drop method for obtaining protozoa-free cultures of bacteria, based on the transfer to a suitable medium of a drop from a protozoan culture which upon microscopic examination revealed no protozoa. On the other hand, he does not describe any direct method for obtaining a bacteria-free culture of protozoa. Jordan (5, p. 469) mentions a method which might prove somewhat tedious—that is, having protozoa pass through concentric rings of dead bacteria on a culture plate until they had no living adhering bacteria. He refers also to Frosch's³ method of separation by means of a sodium-carbonate solution. Richter (8) suggests the use of a high-gelatin medium which would suppress the bacterial growth of liquefying organisms. Biffi and Razzeto (1) give an account of the passage of protozoa through semipermeable filters after a considerable period of time has elapsed.

The writers are in agreement with Biffi and Razzeto regarding the importance of the time element in filtration, since it has been observed that protozoa have been able to work through the pores of a filter in a short time.

In the work under consideration—namely, the separation of flagellates from ciliates—an 8-day-old culture of soil organisms was employed.

¹ From the Departments of Soil Chemistry and Bacteriology, New Jersey Experiment Station, New Brunswick, N. J.

² Reference is made by number to "Literature cited," p. 139-140.

³ Frosch, P. Zur Frage der Reinzüchtung der Amöben. *In* *Centbl. Bakt. [etc.]*, Abt. 1, Bd. 21, No. 24/25, p. 926-932. 1897.

This was prepared by adding 100 gm. of Penn clay loam soil to 1 liter of a 10 per cent hay infusion plus 0.5 per cent of egg albumin, which the writers had previously found to be best adapted to the large and rapid development of protozoa in such soil (6).

The method of procedure was as follows: The numbers of protozoa in the stock culture solution were first counted by the new method described in a previous paper (6) and recorded under classes of (1) flagellates, (2) small ciliates (12 to 20 μ), and (3) large ciliates (25 to 60 μ). No amœbæ developed in the short period of incubation. Ten c. c. of the culture solution were then placed (by means of a sterile pipette) on filter paper, previously sterilized with alcohol, and allowed to filter through for one minute. The protozoan content of the filtrate was then recorded in triplicate and the filtrate incubated for five days at 22° C., in order to allow the excystment of any encysted forms. The filtration and incubation processes were then repeated, if necessary, until all the living protozoa of the desired type had been separated out. The filter paper was used in from one to five different thicknesses (Schleicher and Schüll's No. 589). The results are recorded in Table I.

TABLE I.—Number of protozoa per 10 c. c. of filtrate through varying thicknesses of filter paper

Number of filter papers.	Sample No.	Number of flagellates.	Number of small ciliates, 12-20 μ .	Number of large ciliates, 25-60 μ .	Total.
0 ^a	1	106,250	53,125	42,500	201,875
	2	127,500	42,500	31,875	201,875
	3	85,000	21,250	81,875	188,125
	Average.....	106,250	38,958	52,083	197,292
1.....	1	63,750	53,125	0	116,875
	2	63,750	31,875	0	95,625
	3	74,375	31,875	0	106,250
	Average.....	67,293	38,958	0	106,246
2.....	1	53,125	31,875	0	85,000
	2	53,125	21,250	0	74,375
	3	73,750	21,250	0	95,250
	Average.....	60,416	24,742	0	85,208
3.....	1	53,125	10,625	0	63,750
	2	53,125	10,625	0	63,750
	3	63,750	10,625	0	73,750
	Average.....	56,666	10,625	0	67,083
4.....	1	10,625	0	0	10,625
	2	10,625	0	0	10,625
	3	10,625	0	0	10,625
	Average.....	10,625	0	0	10,625
5.....	1	None.	None.	None.	None.
	2	None.	None.	None.	None.
	3	None.	None.	None.	None.
	Average.....				

^a Stock protozoan solution.

It will be observed from Table I that the large ciliates are not able to pass through the filter paper at all, which fact is in agreement with the experience of Russell and Hutchinson (9, 10). The noteworthy feature, however, is that the number of small ciliates decreases rapidly in increasing the thicknesses of the filter paper from two to four. Thus, with four thicknesses of filter paper all of the ciliates found in the solution employed were separated from the flagellates. Likewise it was a simple matter to separate the small from the large ciliates. In this way it becomes possible to employ mass cultures of flagellates, small ciliates, or large ciliates, as may be necessary in the problems indicated at the outset.

In an effort to determine the effect of filtration on the separation of soil protozoa from bacteria, a bacterial count was made of the stock-culture solution previously employed, known to contain soil micro-organisms. Ten c. c. of this solution were then filtered through five thicknesses of sterilized (with alcohol) filter paper (S. & S. No. 589). The residue on the filter paper, consisting of all of the protozoa originally present, together with some adhering bacteria, was then plated out on Lipman and Brown's (7, p. 132) synthetic agar. The bacterial count showed that 90 per cent of the bacteria had passed through the filter paper (after making due deduction for contamination from the air by exposing agar plates for the same length of time as was necessary for filtration), thus leaving the protozoan residue comparatively free from bacteria.

This method in all probability would not allow complete separation of the protozoa from the bacteria. Consequently the work was not carried out any farther. However, this method, because of its rapidity and simplicity, might prove of value in investigations concerned with the effect of protozoa on mixed but not on pure cultures of bacteria.

While these preliminary experiments do not warrant any definite conclusions, they are, nevertheless, indicative of some of the difficulties which the soil protozoologist encounters.

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No. 4

EFFECT OF TEMPERATURE ON MOVEMENT OF WATER VAPOR AND CAPILLARY MOISTURE IN SOILS

By G. J. BOUYOUKOS,

Research Soil Physicist, Michigan Agricultural Experiment Station

INTRODUCTION

An investigation of the influence of temperature on the various physical processes in the soil was undertaken by the writer at the Michigan Agricultural Experiment Station. One of the phases of this investigation, the effect of temperature on the movement of water vapor and capillary moisture in soils, will be considered in the present paper.

MOVEMENT OF MOISTURE FROM WARM TO COLD COLUMN OF SOIL OF UNIFORM MOISTURE CONTENT

A rise of temperature decreases both the surface tension and the viscosity of water to the extent shown by the data in Table I.

TABLE I.—*Relation of temperature to the surface tension and viscosity of water*

Temperature.	Surface tension.	Viscosity.
°C.		
0	100.00	100.00
10	97.96	73.32
20	94.32	56.70
30	91.62	45.12
40	88.46	36.96
50	85.52	30.17

It will be noted that the degree of diminution with rise in temperature is considerably greater in the case of viscosity than in that of surface tension.

During the warm part of the year the soil at the upper depths maintains a rather marked temperature gradient which reverses itself between day and night to the depth that the diurnal amplitude of temperature oscillation extends. This diurnal change of temperature gradient occasions an alteration in surface tension and viscosity of the soil moisture, the amount depending upon its variation at the different depths. Since capillary action is said to depend upon surface tension and facility of movement upon viscosity, there should occur an up-

ward and downward movement of moisture as the temperature gradient changes diurnally. During the day, for example, the temperature of the soil is highest at the surface and diminishes with depth; the surface tension and the viscosity of soil moisture are lowest at the surface and rise with depth; consequently, the movement of moisture should be downward. During the night the reverse is true; the soil temperature is lowest at the surface and increases with depth; the surface tension and the viscosity of the soil water are greatest at the top and diminish downward with increase of temperature; hence, the water translocation should be upward.

These considerations are *a priori* deductions from the laws of surface tension and viscosity in their relation to temperature. Whether or not

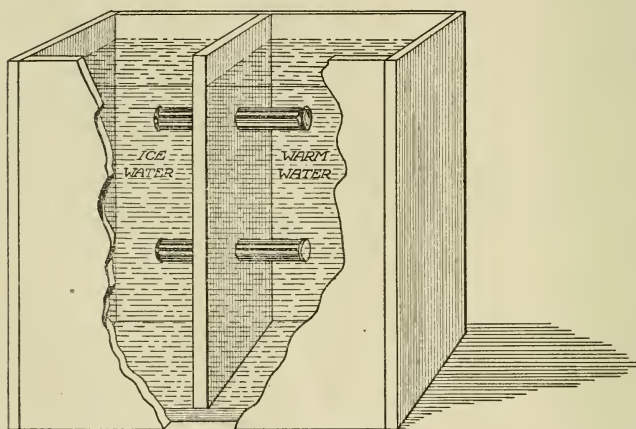


FIG. 1.—Apparatus for determining thermal translocation of soil moisture when the column of soil lay horizontally.

they are valid, however, has heretofore not been known, since there appear to be no experimental data bearing directly upon the subject.

With the object of obtaining this important and much desired information, an investigation of the problem was undertaken. The general method of procedure consisted of placing soils of different but uniform moisture content in brass tubes 8 inches long and $1\frac{1}{2}$ inches in diameter, closing both ends with solid rubber stoppers, and keeping one half of the soil column at a high temperature and the other half at a low temperature for a certain length of time, then determining the percentage of moisture of the two columns and attributing any difference in water content to thermal translocation. There were only two amplitudes of temperature employed, 0° to 20° and 0° to 40° C.—i. e., one half of the soil column was kept at 0° and the other half at 20° and

40° C. For producing these temperature amplitudes wooden boxes were used which contained melting ice and warm water in separate boxes or compartments the temperatures of which were maintained constant by the addition of ice and hot water, respectively.

The movement of moisture from warm to cold soil was studied in two different ways: (1) When the column of soil lay horizontally and (2) when it stood vertically. For the first case, the wooden boxes used were 22 inches long, 10 inches wide, and 20 inches deep, having wooden partitions in the center which contained perforations of the size to fit the tubes (fig. 1). One compartment contained melting ice and the other water at the required temperature. To prevent any exchange of water between the two compartments, the edges of the partition and the

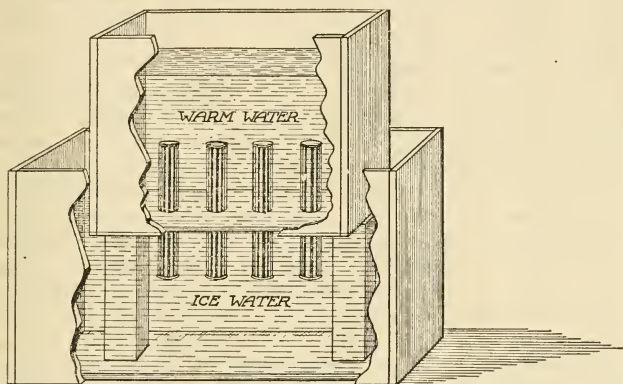


FIG. 2.—Apparatus for determining thermal translocation of soil moisture when the column of soil stood vertically.

holes through which the tubes passed were made water-tight by means of paraffin.

For the second study, the employment of two boxes was necessary (fig. 2). One box, which contained melting ice, was 24 inches long, 10 inches wide, and 13 inches deep. The other box, which contained water at the desired temperature, was 13 inches long, 7 inches wide, and 11 inches deep, and was placed inside the first box. The bottom of the small box was supplied with holes the exact size of the tubes, which were then placed in the holes and the crevices surrounding them sealed with melted paraffin to make the small box waterproof. The inner box was then put upon supports in the large box and was filled with water kept at the desired temperature. The outer box was filled with ice up to and touching the bottom of the inner box. All the boxes were well insulated, and since they were big and contained large volumes of water, the temperature could be kept to within small variations for long

periods. The water was stirred occasionally to maintain uniformity of temperature throughout its mass.

The temperature amplitudes employed are within the upper limit of the diurnal amplitudes of temperature at the upper depths in the soil, but they are too high for the range of temperature that exists at any one time between the various adjacent depths.

The duration of each experiment was about eight hours. This time limit was calculated to represent approximately the length of period that the day and night soil temperature gradient is most marked.

The effect of temperature on the movement of moisture in soils of uniform moisture content was investigated in five diverse classes of soil: Miami light sandy loam, Miami heavy sandy loam, Miami silt loam, Clyde silt loam, and Miami clay. Each soil contained a large number of different moisture contents. These various moisture contents in each soil ranged from very low to very high.

To procure a very uniform moisture content throughout the soil column, each soil, after it was moistened to the desired degree, was passed through a sieve and then mixed thoroughly. It was then placed in the tubes and packed uniformly by allowing the tubes to fall in a vertical position from a certain height a definite number of times.

At the end of each experiment the warm column was separated from the cold column of soil by means of a spatula. This was done by drawing out all the soil from that warm section of the tube which extended up to the plane of the partition and allowing for the cold column all the soil that was contained in that cold section of the tube up to the other plane of the partition, and also that portion of the soil contained in the tube under the hole of the partition. This last part of the soil was accorded to the cold column of soil because its temperature is intermediate between the opposite temperature extremes, and it was desired to make the lines of demarcation between the two columns of soil as prominent and distinct as possible. The moist soils were dried in an electrical oven for about 20 hours at a temperature of 105° C., and the percentage of moisture content was calculated on the dry basis. The weights were always determined on a sensitive chemical balance.

The fact has been mentioned that the movement of moisture from a warm to a cold column of soil was studied in two different ways: (1) When the column of soil lay horizontally and (2) when it stood vertically. The data obtained from both series of experiments show that if the same percentages of moisture were employed practically the same results would be obtained, no matter whether the soil columns remained in the horizontal or vertical position. For the sake of brevity and simplicity of presentation, therefore, only the results of the series of experiments wherein the soil column was held in the vertical position will be presented here. These experimental data, together with their diagrammatic representations, are submitted below. Table II gives the

various moisture contents of the different soils and the percentage of moisture moved from the column of soil at 20° to the column of soil at 0° and from the column of soil at 40° to the column of soil at 0°. The percentage of moisture moved represents the difference between the percentages of moisture found in the cold and the warm columns of soils, respectively, at the end of the experiment; at the beginning of the experiment the moisture content was the same in both columns of soil. Figure 3 represents these data in a graphical form.

TABLE II.—Movement of moisture from a warm to a cold column of soil of uniform moisture content

Kind of soil.	Percentage of moisture in soils.									
Sandy loam:										
Beginning of experiment.....	2.29	3.86	6.45	7.50	8.48	9.95	10.94	13.75	15.96
Movement from 20° to 0° C.....	.102	.296	.792	.900	.530	.520	.466	.340	.100
Movement from 40° to 0° C.....	.410	1.064	1.97	2.882	1.715	1.467	1.30	.97	.30
Heavy sandy loam:										
Beginning of experiment.....	4.20	6.52	9.08	10.42	12.43	14.02	16.03
Movement from 20° to 0° C.....	.160	.631	.930	.721	.582	.491	.21
Movement from 40° to 0° C.....	.59	1.75	3.02	2.40	1.98	1.40	.42
Silt loam:										
Beginning of experiment.....	4.29	8.06	9.76	11.28	14.441	15.95	17.63	19.30	21.42	23.51
Movement from 20° to 0° C.....	.138	.736	1.024	1.180	1.190	1.10	.85	.48	.35	.21
Movement from 40° to 0° C.....	.471	1.98	2.65	3.276	3.68	3.58	2.60	1.75	1.02	.45
Clyde silt loam:										
Beginning of experiment.....	7.56	12.51	14.98	17.59	18.80	21.55	22.76	29.98	34.57
Movement from 20° to 0° C.....	.122	.46	.89	.96	1.07	.99	.83	.62	.20
Movement from 40° to 0° C.....	.405	1.72	2.07	2.45	3.27	2.82	2.30	1.36	.51
Clay:										
Beginning of experiment.....	9.70	18.38	19.29	20.69	22.98	29.88
Movement from 20° to 0° C.....	.248	.72	.99	.73	.70	.681
Movement from 40° to 0° C.....	.672	2.60	3.29	2.50	2.12	1.88

The foregoing data present many important and remarkable facts. First of all, they show most emphatically that the *a priori* prediction regarding the thermal movement of moisture as deduced from the laws of surface tension and viscosity in their relation to temperature is not strictly realized. According to these laws, the amount of water moved from a warm to a cold column of soil should be the same for all moisture contents, provided the soil mass exerts no influence upon water; inasmuch, however, as the soil does exert an adhesive force upon water, the thermal translocation of moisture should increase with a rise in water content. Instead, the percentage of water moved from a warm to a cold column of soil at both temperature amplitudes increases regularly and rapidly with an increase in moisture content in all the different types of soil until a certain moisture content is reached, and then it begins to decrease with a further rise in the percentage of water. The results then plot into a parabola, with a maximum point instead of a straight line. This maximum point of water thermal translocation is significant in at least two ways: (1) It is quantitatively about the same for all classes of soil and qualitatively the same for both amplitudes of temperature; and (2) it is attained at entirely different moisture contents in the various soils and at a comparatively low percentage

of moisture. On referring to the data in Table II it will be seen that the maximum thermal water transference at the amplitude of 20° C. is 0.90 per cent for light sandy loam, 0.93 for heavy sandy loam, 1.19 for silt loam, 1.07 for Clyde silt loam, and 0.99 for clay; at the temperature amplitude of 40° it is 2.88 per cent for light sandy loam, 3.02 for heavy sandy loam, 3.68 for silt loam, 3.27 for Clyde silt loam, and 3.29 for clay. It should be noted that the percentage of thermal

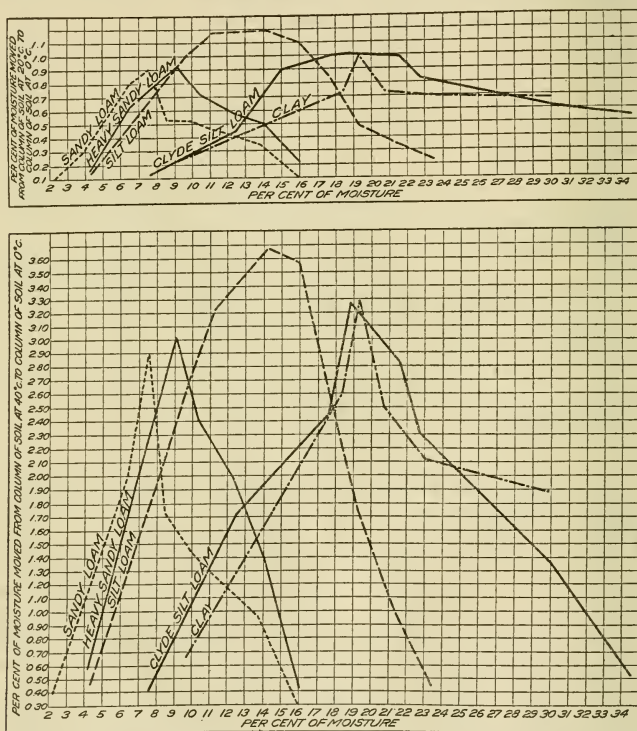


FIG. 3.—Curve showing the movement of moisture from a warm to a cold column of soil of uniform moisture content.

motion of water increases more than proportionally with temperature. The temperature of 40° , for instance, is only twice as great as 20° , while the percentage of moisture moved is three times greater in the former case than in the latter. The water content of the various soils at which the maximum thermal translocation occurs is 7.50 per cent for light sandy loam, 9.08 for heavy sandy loam, 14.21 for silt loam, 18.80 for Clyde silt loam, and 19.29 for clay.

Obviously, then, the maximum thermal water movement depends upon a definite condition of moisture of any particular soil; a deviation from this definite degree of moisture in either direction causes a decrease in thermal movement of water. Since this definite percentage of moisture at which the greatest quantity of water is able to move from a warm to a cold column of soil appears to be a specific constant or characteristic of the various soils, it is proposed to designate it as "thermal critical moisture content." A thermal critical moisture content may be defined, then, as that percentage of moisture in soil which allows the greatest amount of water to move from warm to cold soil at any amplitude of temperature.

A further examination of the preceding experimental data shows that the thermal movement of moisture is extremely sensitive to the amount of water present in a soil. It will be noted that by increasing or decreasing the percentage of soil water by small degrees, the thermal movement varies very markedly in either direction. From this it follows that the thermal critical moisture content must be quite definite, and in order to obtain it absolutely, the percentage of soil moisture near the point of maximum thermal movement must be increased by small amounts. This applies especially to the light sandy soils, in which the sensitiveness appears to be more marked and the range more limited. If the increase in percentage of moisture content took place in this soil by 0.1 instead of 1.0 per cent, the maximum thermal translocation would probably have been as high as that of the other soils. It is possible, however, that the value obtained is about the upper limit for this soil and consequently for all soils of its type.

The diminution of the thermal translocation of water with a decrease in moisture content from the point of thermal critical moisture content might be anticipated, but the decrease of water movement with further increase of moisture content after the point of thermal critical moisture content was not expected. Indeed, it was at first thought that the movement would be greater at the highest moisture content because there would also occur a gravitational movement. When soils contain as high as 35 and 30 per cent of moisture, as did the Clyde silt loam and the clay, respectively, and when one half of their column is kept at 40° and the other at 0° C. for eight hours, such expectation as the above is not at all unnatural. Instead, the water movement at these highest moisture contents is very low and in descending order and the cessation of diminution is not as yet reached. These results go to show, then, in a most striking manner that the soils possess a very great attraction for water and that their requirements for water to satisfy their attractive forces before free movement of water can take place are, indeed, high. Until the point is reached where gravitational movement occurs, the moisture in the soil is held by a force of great magnitude.

Now, the next question is, How may this peculiar thermal translocation of water be explained? What are the causal agents which bring it about?

As already stated, it is not entirely due to the surface tension and viscosity of the soil water, for if that were the case then the movement should have followed a different course. If the soil exerted no adhesive force for water, the amount of moisture moved from a warm to a cold column of soil should be the same for all moisture contents, provided the force of gravity is eliminated for any particular amplitude of temperature. But since the soil does exert a strong adhesive force for water, the thermal motion of water should follow a straight line with rise in moisture content for any given difference in temperature. Instead, the results plot into a parabola. Evidently there must be another explanation for the phenomena.

The best explanation suggested appears to be founded upon the following four assumptions: (1) The soil possesses an attractive power for water and holds it with a great adhesive force; (2) these attractive and adhesive forces decrease with increase in temperature; (3) the surface tension or cohesive power of the liquid also diminishes with rise in temperature; and (4) the force due to the curvature of the water films between the soil grains, which are known as capillary films, decreases with increase of water content.

All these four assumptions appear to be correct. The validity of the third and fourth is generally recognized and consequently needs no further discussion. The validity of the first is also universally accepted: That the soil possesses an attractive power for water can hardly be denied; that the soil holds the water with a great adhesive force is evidenced by the great difficulty experienced in attempting to separate the one from the other. Indeed, this adhesive force is so great that no method as yet has been devised either to execute a complete separation of the two components or to measure with any degree of precision its magnitude. The researches of Lagergren (8),¹ Young,² and Lord Rayleigh (10) indicate, however, that this force may be of an order of magnitude from 6,000 to 25,000 atmospheres.

The great attractive and adhesive forces which the soil exerts upon water are further illustrated by the researches of Briggs and McLane (3) on the moisture equivalent and by those of Briggs and Shantz (4) on the wilting coefficient of plants. By whirling wetted soils in a rapidly revolving centrifuge fitted with a filtering device in the periphery and developing a force equivalent on the average to 3,000 times the attraction of gravity, Briggs and McLane found that some clay soils would still contain about 50 per cent of water. The studies of Briggs and McLane on the wilting coefficient of plants show that plants would wilt and die in clay soils even

¹ Reference is made by number to "Literature cited," p. 172.

² Cited by Minchin, G. M. *Hydrostatics and Elementary Hydrokinetics*, p. 311, London, 1892.

when the moisture content was still about 30 per cent. As the water content increases, these attractive and adhesive forces decrease.

Of all the four assumptions the correctness of the third—namely, that the attractive and adhesive forces decrease with temperature—may be doubted by many and challenged by a few. The theoretical and experimental evidences, however, are overwhelmingly in its favor. According to the law of kinetic energy, the attractive and adhesive forces of solids for liquids and gases or vapors should decrease with rise in temperature. The investigations upon the absorption of gases and vapors at different temperatures show this to be the case. The work of De Saussure (11) and Von Dobeneck (6) upon the absorption of gas by different solid materials, and the researches of Knop¹ and Ammon (1) upon the absorption of water vapor by soil, seem to show conclusively that the absorptive power of diverse solid materials for gases and water vapor decreases with increase in temperature. The only evidence which is contrary to the above is that obtained by Hilgard (7, p. 198) on the absorption of water by dry soils from a saturated atmosphere. Hilgard's results show that the absorption of water vapor by soils increases with rise in temperature. The results obtained by the several investigators mentioned, as well as new evidence which will subsequently be presented, tend to throw considerable doubt on the correctness of Hilgard's data. Hence, it can safely be asserted that the third assumption is correct.

Bearing these postulates in mind, the phenomena of thermal water translocation observed may be explained as follows: The soil with the lowest moisture content holds the water with a force of great magnitude. When the temperature of a column of this soil is uniform throughout, the adhesive and attractive forces are at an equilibrium. When one half of this column of soil is heated to 40° and the other half to 0° C., this equilibrium is disturbed. The attractive and adhesive forces of the soil for water and the cohesive power or surface tension of the soil water are decreased in that portion of the soil column which is maintained at 40° and increased to a corresponding magnitude in that portion of the soil column which is kept at 0° C. The cold column therefore exerts a pull and draws water from the warm column in amount depending upon the quantity that the latter is willing to give up. Since the soil possesses a great attraction for water, which attraction varies with the diverse classes of soil, and inasmuch as this attractive force is not satisfied at the low moisture content, the warm soil parts only with a small amount of its water. Hence, the amount of water moved from the warm column to the cold column of soil is small.

At the next higher moisture content the attractive power of the soil for water is further satisfied and the total water content is held with less force. When a column of this soil is kept at the same amplitudes of temperature as above, the decrease and increase of the adhesive

¹ Cited by Johnson, S. W. *How Crops Feed*. p. 164. New York [1870].

and cohesive forces, due entirely to temperature, between the warm and cold columns of soil are equal in amount, as in the soil with the lowest moisture content. Water, therefore, tends to move from the warm to the cold soil. Inasmuch as the attraction of the soil has been further satisfied and the water films further thickened, the pull of the cold soil, due only to the attractive forces of the soil for water, is decreased; on the other hand, the ease with which the warm soil gives up moisture is increased. The result is that even though the total effective pull (composed of the increased surface tension of water, the increased attractive adhesive forces of soil for water, and the force of the curvature of the capillary films) of the cold soil with the high moisture content is less than that of the soil with low moisture content, the greater ease with which the warm soil with high water content parts with moisture enables the reduced effective pull to draw more water from the warm to the cold side. As the moisture content of the soil is continually increased, its attractive power is satisfied and the curvature of the capillary films decreased correspondingly. The total effective pull of the cold column of soil is continually decreased, but the ease with which the warm column of soil gives up moisture is also continually increased, so that the thermal translocation of water is constantly increased with rise in moisture content.

Finally, a degree of moisture content is reached in which the effective pull of the cold column of soil is able to extract the greatest amount of water from the warm column of soil. This degree of water content is the thermal critical moisture content. At this point the attractive power of the soil for water is considerably satisfied but is far from being entirely appeased; the total effective pull of the cold column of soil is also considerably less than that of the preceding columns of soil, but the warm column yields water to this pull with such ease that there occurs a maximum thermal water translocation. Inasmuch as the water-attractive power is different for the various kinds of soils, this thermal critical moisture content is of necessity also different. After this thermal critical moisture content is reached, the effective pull of the cold column of soil is further decreased with a continued increase of moisture content. And although the willingness of the warm column of soil to part more readily with moisture is also increased, yet the pull of the cold column of soil is not sufficiently strong to draw it; consequently the thermal movement of water commences to decrease and continues to diminish very regularly and gradually with a continued increase in moisture content. When the highest percentage of water is reached, the warm soil is very willing to part with a very large amount of water, but since the effective pull of the solid soil is reduced almost to a minimum, only a small amount of moisture is drawn from the former to the latter.

The degree of moisture of the different soils could not be further increased, on account of the difficulty of sifting them, and consequently it

can not be stated with certainty whether the thermal movement of water would become zero at a still higher moisture content. From the theoretical point of view, however, it should not become zero, because the pull due to the surface tension of water alone is not affected by increase of moisture content, but remains constant. The portion of pulling force which is decreased constantly with a rise in moisture content is that pertaining to the attractive power of soil for water and to the curvature of the capillary film. At or near the point of saturation the pulling power due to these two factors is probably zero; at this point the soil may be considered to be passive. Any thermal movement of water that takes place at or near the point of saturation is to be attributed to the surface tension of the soil water. If this assumption is correct and if the percentage of moisture moved at the highest moisture contents employed is to be considered as a measure of the amount of thermal translocation due to surface tension of water alone, it will be found that the quantity due to this force is very small indeed.

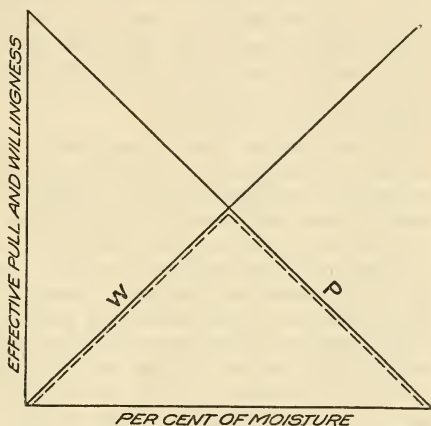


FIG. 4.—Diagram illustrating the cause and mechanism of moisture movement from a warm to a cold column of soil of uniform moisture content.

As will be seen from the experimental data, the percentage of moisture moved at both amplitudes of temperature is reduced to an insignificant value at the highest moisture contents.

The foregoing exposition as to the cause and mechanism of the phenomena of thermal water translocation will probably be made clearer by figure 4. This diagrammatic representation, however, by no means pictures the real cause and mechanism absolutely and accurately, but it will serve, it is believed, to make clearer what has already been said.

Let the abscissa represent the effective pull of the cold column of soil and the willingness of the warm column of soil to part with water at a different moisture content, and let the ordinates represent the different percentages of water contained by the soil. By plotting the effective pull and willingness against the moisture content it will be seen that the effective pull decreases and the willingness increases with a rise in moisture content. At the point where the two lines cross probably occurs the

maximum thermal translocation of water. After this point of intersection the willingness of the warm soil to give up water is large, but since the effective pull is being reduced to a minimum the water is not moved. If a parabola is now drawn along the lines WP, with its maximum value at the point of intersection, then this theoretical curve will agree almost perfectly with the real one in figure 3.

The serious fault with the above illustration (fig. 4) is that the total effective pull tends to become zero, and theoretically this should not be the case, because while the pull due to the attractive power of the soil for water and to the curvature of the capillary films will ultimately become zero, the pull due to the increased surface tension of the soil water should not become zero, but should remain the same for all moisture contents. Hence, figure 4 illustrates more correctly only the thermal translocation of the water as due to all the other forces except the surface tension of water.

The next important question to consider is the mode and amount of thermal translocation of water in field soils as suggested by the foregoing laboratory experimental data. Under field conditions the soil moisture exists practically always in a gradient form. As the water content tends to decrease upward from the water level, the forces due to the curvature of the capillary film and to the attractive power of the soil for water increase correspondingly; consequently the pull is upward. The soil temperature also exists in a gradient form, but this reverses itself diurnally and therefore modifies these pulling forces. During the day the temperature at the upper depths is higher than that below; the attractive and adhesive forces of the soil for water and the surface tension of water are decreased, so that the total upward effective pull is diminished correspondingly. Inasmuch as the temperature below is less than that above, the effective pull due only to the increased attractive and adhesive forces of the soil for water and to the surface tension of the soil water should occasion a downward movement of moisture. Since, however, the water-attractive forces of the soil below are more satisfied than those of the soil above, the downward pull due only to the attractive adhesion and surface tension as increased by a lower temperature is very small in comparison with the upward pull. Hence, during the day the moisture movement is upward. During the night nearly all of the above forces act in a parallel direction and favor an upward movement. Therefore, the thermal movement of moisture in soils is always upward and never downward.

The extent to which moisture will move during the night from the warmer soil below to the colder soil above will depend (1) upon the soil temperature gradient—that is, upon the difference in temperature of the various adjacent depths—and (2) upon the gradient or amount of moisture content at the various depths. In the preceding series of

experiments the temperature amplitudes of 20° and 40° C. were employed. In nature, however, so large and sharp variations in temperature between adjacent depths never occur during the night; they do occur, however, at the upper depths between day and night. Soil-temperature investigations which are being conducted at this Station show that in the early morning, when the temperature gradient is most marked, the temperature of the bare mineral soils increases sometimes in the summer and fall at the average rate of about 2° or 3° for each inch of depth down to about 4 inches, and then this rate becomes less. In cropped soils, where the temperature remains more constant, this rate of increase of temperature with depth is still less. Hence, the amount of thermal translocation of water that would occur during a single night would be very small. On the other hand, the maximum thermal translocation of water obtained in the preceding series of experiments was procured from a column of soil with uniform moisture content. As will be shown subsequently, there is no doubt whatever that this maximum thermal translocation of water in the various soils would have been far greater if the moisture content of the cold column was less than that of the warm column of soil. In nature, as already mentioned, the moisture exists in a gradient form; consequently the movement of water is upward and the forces of the factors which cause this upward movement are increased during the night. Therefore, while the amount of thermal translocation of water during a single night in soils under field conditions may not be as great as that obtained in the foregoing series of experiments, yet it will be quite appreciable; and since the process is repeated, the sum of the translocation for all the nights during the vegetative season will probably be considerable.

The moisture content at which the maximum thermal translocation of water occurs, or what has been designated as the thermal critical moisture content, is very significant and needs further consideration. It would be of very great interest to know, for instance, the thickness of the water film around the particles at this degree of moisture. This thickness could be calculated if all the soil grains were solid and spherical. The particles of the soils used, however—and these are the commoner types of agricultural soils—are neither spherical nor solid. Nearly all the particles in agricultural soils can be said to be irregular in shape. Some of them are solid and enveloped with a colloidal coating; others are compound aggregates, or “crumbs,” and are porous; and still others, mainly of the peat nature, are of a sponge structure and are necessarily porous. The particles of a soil or soils may be classified under two categories: (1) Particles which are solid and have only an external surface and (2) particles which are partly or wholly porous and possess both an external and internal surface. In the solid and cleaned surface particles the water film is spread over the surface, but the film of water

envelops theoretically the whole external surface of the solid particles coated with colloids, or the mineral floccules and the organic particles; and water also permeates their internal surface. The single solid mineral grains, which may compose the compound particles, may be cemented together in a way analogous to that found in a piece of sandstone, in which case the water exists only in the interstices and not as a complete film around each particle. Furthermore, whether the soil grains are solid or spherical or compound and porous the water film is not uniform in thickness over the entire inner surface of the soil mass, but thickens more at the capillary angles between the particles.

In view of these considerations, therefore, it was considered useless to attempt to compute the thickness of the film, as many investigators have done. Furthermore, in view of the nature of the soil particles, as discussed above, it does not appear strictly proper to define the capillary water in the soil as a thin film overspreading the particles and thickened into a waistlike form at their points of contact. Hence, a new definition of capillary water is needed.

If we are to accept the theory which has been used to explain the foregoing phenomena of thermal translocation of water, that the soil possesses a very great attraction for water, that this attractive force is different for various soils, that it decreases with a rise in moisture content, and that it is completely satisfied at a considerably high moisture content, then our present views concerning the movement of capillary water in moist soils need modification. The present theory regarding the capillary movement of water consists of an analogy from the rise of water in capillary tubes. The interstitial spaces of a soil mass are considered as forming channels analogous to capillary tubes and are often designated as bundles of capillary spaces. The capillary water is believed to exist as surface films around the particles and as capillary films in the capillary spaces between the particles, and its movement is said to depend entirely upon the curvature of the capillary films. When a dry soil, for instance, is well moistened and brought to equilibrium, the water films are thick and the curvature of the capillary films small, and there will be no further capillary attraction of water if this soil is brought in contact with water. If this soil is allowed to dry at the top, the surface films become thinner and the force of the capillary films increases in direct ratio with their degree of curvature; hence, there will be a pull of water from the thicker surface films and less curved capillary films below toward the surface.

It is obvious that with this theory of capillary movement of water the whole cause of the capillary movement of water in a moist soil is attributed to the curvature of the capillary films between the particles, and the moist soil is considered as being passive, inactive, and exerting no influence whatever upon the movement of water. Indeed, Briggs

and Lapham (2), in trying to explain the differences in capillary action in dry and moist soils, make the following statement:

In a moist soil, however, we have quite another condition. A film of the liquid covers all the surfaces of the soil grains. Since this film, once established, is maintained in a saturated atmosphere, it follows that the solid air and solid liquid surface forces no longer play any part in the capillary movement, which is produced entirely by the air liquid surface force and is opposed only by the weight of the liquid column.

In view of this general belief, Briggs, as well as other investigators, has tried to alter the properties of the soil water by increasing its surface tension, etc., with the object in view of increasing its capillary action.

If it were true that as long as a thin film of water is maintained in a damp or slightly moist soil the soil material itself exerts no longer any influence upon the movement of capillary water, then the preceding theory might be true. But we have seen in postulate 1 (p. 148) that the soils, and especially those rich in colloidal material, possess a very great attractive power for water, that this attractive power is satisfied only at a considerably high moisture content, that as long as it is not satisfied the soils will continue to take up water, and that they hold the water with a force of great magnitude. In view of the considerations presented in this postulate, and in view of the fact that the preceding thermal movement of water appears to be controlled by the attractive forces of the soil for water, it seems wrong to consider the soil material in moist condition as a static, passive, inactive, and irresponsive skeleton upon which the liquid plays its rôle. The solid material in moist condition short of saturation is dynamic and not static in respect to moisture movement. Hence, the capillary movement of water should not be attributed entirely to the forces exerted by the curvature of the capillary films, but also to the forces exerted by the unsatisfied attractive power of the soil for water. When a moist soil, therefore, begins to lose water at the surface, two effects are produced: (1) The attractive forces of the soil for water are increased and (2) the curvature of the capillary films is increased. Both of these effects exert a pull on the moist soil below and tend to draw water to the surface. As to which one of these two forces exerts the greatest pull it is impossible to say, because there is no way of measuring them. It is certain, however, that the force resulting from the attractive power of the soil for water must be very considerable, and probably it is the predominant of the two.

It might be argued that the preceding phenomena of thermal translocation of water could be explained entirely by the film theory without having to resort to the conception of the attractive forces of the soil. Such contention, however, can not be maintained, first, because it can not be conceived that the tension of the capillary films is operative and effective at such high moisture contents employed and, second, because the fact remains, nevertheless, that the soil exerts a pull owing to its

attractive forces for water, as has been abundantly proved. Furthermore, if it is maintained that the attractive forces of the soil for water are satisfied as soon as the soil is merely damped, then why should the soil hold additional large amounts of water with such a great force that it is impossible to extract it with mechanical means? It seems reasonable, therefore, to believe that if the soil holds large amounts of water with a great force, it should attract or absorb it with a force of equal magnitude.

MOVEMENT OF MOISTURE FROM A MOIST AND WARM COLUMN TO A DRY AND COLD COLUMN OF SOIL WITH AN AIR SPACE BETWEEN THE TWO COLUMNS

In the preceding section the thermal translocation of water was considered as occurring as water-film phenomena. There is still another way in which this thermal movement of moisture might take place: By vaporization and condensation of soil water from a point of high temperature to a point of low temperature. It is well known that water undergoes a transformation into the vapor state upon the application of heat, and the quantity of liquid vaporized increases with a rise in temperature. One of the remarkable characteristics of aqueous vapor is its sensitiveness to heat, changing from a gaseous to a liquid state, and vice versa, with very small variations in temperature. An excellent paradigm of this latter fact is the relative humidity of the air at different temperatures.

Since the temperature gradient of the soil reverses itself during the night—that is, it increases with depth—it is believed that there is a rising of vapor or moist air from the warmer soil below to the colder soil above, where the moisture is condensed. As a manifest proof of this theory, the morning dew is cited. It is concluded, therefore, that a large part of the water movement in soils is due to this process.

There appear to exist no direct experimental data as to whether or not there really is a translocation of moisture in soil at night, due to upward movement of the moist warm air and the condensation of its moisture at the cold soil above. Practically all of our present knowledge upon the subject consists of theoretical deductions from practical observations.

With the object of obtaining experimental evidence upon the subject the following investigation was performed. Into brass tubes 8 inches long and $1\frac{1}{2}$ inches in diameter was placed moist soil at one end and dry soil at the other and the two columns separated by an air space. This air space was one-fourth of an inch in height and $1\frac{1}{2}$ inches in diameter and was produced by placing between the two columns of soil a ring of cork, the two sides of which were closed with wire gauze that acted as supports of the two soils and prevented their particles from coming in contact. The tubes were then placed horizontally in the boxes shown

in figures 1 and 2. That part of the tubes which contained the moist soil was kept at 20° and 40° and the part which contained the dry soil was maintained at 0° C. The experiment was allowed to run about eight hours. If during this period the dry and cold soil gained any moisture, it obtained it by the condensation of vapor which was produced at the warm and moist soil. Since the dry soil possesses a high absorptive power for water, it was assumed that it abstracted the vapor from the air space and that this air space was thus prevented from attaining an equilibrium. Five different classes of soil were used: Quartz sand, Miami light sandy loam, Miami silt loam, Clyde silt loam, and Miami clay. The moisture contents employed for each soil were three: Low, medium, and high. The percentage of moisture moved from the warm and moist column of soil to the cold and dry column of soil represents the difference between the percentages of moisture found in the dry soil at the beginning and end of the experiment. The results obtained are presented in Table III.

TABLE III.—*Movement of moisture from a warm and moist column of soil to a cold and dry column of soil, with an air space between the two columns*

Kind and temperature of soil.	Percentage of moisture in soil.		
Quartz sand:			
At beginning of experiment.....	2.90	6.83	13.52
Movement from moist column at 20° to dry column at 0° C.....	.051	.046	.048
Movement from moist column at 40° to dry column at 0° C.....	.286	.280	.294
Sandy loam:			
At beginning of experiment.....	7.23	10.27	15.82
Movement from moist column at 20° to dry column at 0° C.....	.0238	.0313	.0246
Movement from moist column at 40° to dry column at 0° C.....	.211	.253	.223
Silt loam:			
At beginning of experiment.....	9.16	14.52	16.40
Movement from moist column at 20° to dry column at 0° C.....	.024	.033	.0273
Movement from moist column at 40° to dry column at 0° C.....	.278	.273	.288
Clyde silt loam:			
At beginning of experiment.....	9.85	15.51	22.39
Movement from moist column at 20° to dry column at 0° C.....	.028	.031	.040
Movement from moist column at 40° to dry column at 0° C.....	.16	.22	.28
Clay:			
At beginning of experiment.....	10.77	15.36	20.35
Movement from moist column at 20° to dry column at 0° C.....	.08	.06	.09
Movement from moist column at 40° to dry column at 0° C.....	.18	.36	.26

The results in Table III show the most surprising fact that the amount of moisture moved from the moist and warm column of soil to the dry and cold column of soil by vapor is very insignificant. It will be seen that at the temperature amplitude of 40° the quantity of moisture moved is only about 0.25 per cent, and at the amplitude of 20° the value is only about 0.035 per cent. In comparison with the results of Table II, where it is shown that the maximum thermal movement of water at the thermal critical moisture content, when the soil mass is continuous, runs as high as 3.68 per cent in some cases, the above values, due only to vapor movement and condensation, are extremely insignificant.

From these results then it is safe to conclude that the thermal movement of moisture due to distillation is practically negligible, even at such high amplitudes of temperature as 20° and 40° C., which never exist during the night at the different depths on the soil, nor during such a long, continuous period as eight hours. This conclusion is indirectly substantiated by the studies of Buckingham (5) on the loss of soil moisture by direct evaporation from points below the surface. By exposing a surface of water or moist soil to evaporation into a confined space which was in communication with the outside air through a column of soil, Buckingham found that the actual mean rate of loss of water through diffusion of water vapor through soils in still air was very small.

Another noteworthy fact in the foregoing experimental data is that the amount of distillation from moist and warm to the dry and cold column of soil is the same for all moisture contents. This might have been anticipated, since the amount of water vaporized depends principally upon the temperature and is not governed by the amount of water present. On the other hand, if the amount of water present in the soil is extremely small, the water is held by the soil grains with an attraction of great magnitude, causing a lowering of the vapor pressure of the absorbed water film and thereby producing a diminution in the rate of evaporation. Perhaps the water contained in the soil with the lowest moisture content was above the point where this lowering of vapor pressure occurs; and consequently the partial pressure of the vapor in the air space in this soil was the same as in the air space of the soil with the greater moisture contents. Furthermore, the values are so small as to lie within the experimental error, and the method of moisture determination may not be sufficiently sensitive and accurate to show any decreased evaporation by the soils with the lowest moisture content.

In undertaking and performing the foregoing series of experiments it was taken for granted that there really is an upward movement of moist air during the night from the warmer soil below to the colder soil at the surface, where its vapor is condensed. This theory seems to be now very widely accepted, as already stated. The formation of the dew

is attributed by many writers almost entirely to this thermal movement of vapor. Thus, in discussing the subject Hilgard (7, p. 307) states that "dew is formed from vapor rising from the warmer soil into a colder atmosphere, and condensed on the most strongly heat-radiating surfaces near the ground, such as grass; leaves, both green and dry; wood; and other objects first encountering the rising vapor." Farther on he says: "The fact that dew is most commonly derived from the soil could have been foreseen from the other fact, long ascertained and known, that during the night the soil is as a rule warmer than the air above it." Other writers, such as Ramann (9), etc., claim in substance the identical belief.

But really, is there a rising of vapor or warm moist air from the warm soil below to the cold soil above? And is the source of water of the dew ascribable to this soil vapor? During the day the soil receives its heat at the upper surface, and its temperature rises. The heat is conducted downward, and the temperature of the various depths of the soil increases correspondingly. The temperature at the surface continues to increase until a maximum is reached and then begins to decrease. As the temperature increases and moves downward, the soil air expands, and as the volume of the pore space remains constant, it is expelled into the atmosphere. The pressure of the soil air at the different depths tends to be the same at any one time and equal to the atmospheric pressure, provided the communications are ideal. When the temperature at the surface soil is at the maximum, it is generally many degrees higher than that of the air above, amounting sometimes to 30° C. In fact, the air temperature decreases in calm and clear weather with an increase in height at the adiabatic rate of approximately 0.9° per 300 feet. When the temperature of the surface soil and of the air is highest, the atmospheric pressure also tends to be at its minimum, so that the air escapes from the soil with greater facility. After the surface soil attains its maximum temperature and then begins to cool, its air contracts, tends to produce a partial vacuum, and consequently draws air from the atmosphere, so that its pressure will be in equilibrium with that of the latter. The fall of temperature is also conducted downward and proceeds as a wave, and as it descends it causes a diminution in volume at the corresponding depths and therefore produces an inward flow of air. This cold wave, however, is preceded by the maximum temperature wave, which as it proceeds downward causes a further expansion of air, which goes to make up for the decreased volume of air caused by the cold wave following immediately after. The difference in temperature, however, of the soil at any depth immediately before and after the maximum temperature wave is reached is very small, as experiments at this Station show; consequently the expansion and expulsion of air caused by the downward march of the minimum temperature wave is not very appreciable. Hence, as the cold wave proceeds downward and produces a decrease in volume of the soil air, the air that comes to make

up for this decrease, so that an equilibrium of pressure will exist, is mainly from the outside atmosphere. After a certain depth is reached, the maximum temperature wave entirely disappears, and there is no more upward expulsion or movement of air. From now on, as the temperature of the soil is further decreased and the volume of its air diminished correspondingly, the current of flow of air into the soil is entirely from the outside atmosphere. This downward flow of air will continue until the soil temperature begins to rise again and the cycle recommences. When the minimum temperature of the surface soil is reached, it is, as a rule, about the same or slightly higher than that of the air immediately above. The temperature of the air at about this period increases with the height in the same manner as the temperature of the soil increases with depth, which is just the opposite from what it is during the day. This increase instead of decrease of temperature at night with a rise in elevation is called "surface temperature inversion." At this minimum temperature the atmospheric pressure approaches its maximum, and the inward flow of air is thereby facilitated.

All the foregoing facts lead to the enunciation of a general law that during the day, as the temperature rises, the soil air tends to flow outward into the atmosphere, and during the night, as the temperature falls, air from the atmosphere tends to flow inward into the soil. This law diametrically opposes the prevalent theory that during the night there is an upward movement of moist warm air. The above law, however, seems to be borne out by logic and appears to be confirmed by experimental evidence subsequently to be presented. The prevalent theory seems unreasonable; for instance, if it is admitted, which it must be, that the soil air escapes into the atmosphere during the day as the temperature rises, then where and when does the soil obtain its air if it continues to give up air even during the night? It might be argued that it is vapor that is rising to the surface and not air. That is inconceivable in the present case. It is true that distillation would occur if the amplitude of temperature were appreciable and constant, but it has been shown that the temperature of the whole column of soil decreases constantly and that an air current from the cold atmosphere is drawn inward which tends to encounter and oppose any upward movement of vapor rising from any difference in temperature. Moreover, granting for sake of argument that there is a vapor rising from the warmer soil to the colder soil at the surface, the amount would be extremely small—too small to account for the great quantity of dew commonly noted—because the temperature amplitudes of the soil at different depths at night are never very great. In fact, during the spring months, as the temperature of the lower depths continually rises and the trend of the air temperature is upward, the range of temperature between the surface and the lower depths, say 6 inches, is small, usually amounting only to about 2° or 3° C. The greatest differences in temperature at the different depths in the morning occur

in the fall, when the trend of the air temperature is downward and the surface soil temperature continually falls. At this time the variation in temperature between the surface and 6 inches of the mineral soils may be as high as 8° C.

The truth of the matter, however, seems to be that instead of vapor rising from the warmer soil below to the colder soil at the surface, vapor enters the soil from the atmosphere. This is a natural conclusion from the law enunciated that during the day air is exhaled from the soil and during the night air is inhaled from the atmosphere. The amount of moisture that will thus enter the soil will depend upon the quantity of air inhaled and upon its absolute humidity, but, as calculations show, it is extremely small. The water may be abstracted by the dry soil at the surface as the air is drawn in or it may enter unaffected. Thus, it is possible that the moisture lost by the soil during the day by the expulsion of its moist air is partly, if not wholly, regained at night.

What is, then, the source of the water of the dew? The greatest part of it comes from the lower layer of the atmosphere itself by condensation. Some of it comes from the leaves of trees and plants; and a certain amount comes from the soil by capillary and thermal capillary action, as set forth previously.

According to the foregoing consideration, therefore, the notion that "dew is formed from the vapor rising from the warmer soil into a colder atmosphere" is wrong, and those who proposed and adhere to this theory seem to be laboring under a misapprehension of facts.

MOVEMENT OF MOISTURE FROM A MOIST AND WARM COLUMN TO A DRY AND COLD COLUMN OF SOIL AND FROM A MOIST AND COLD COLUMN TO A DRY AND WARM COLUMN OF SOIL

The soil moisture under field conditions exists during the warm period of the year nearly always in a gradient form. During a long drought even the upper surface dries out, either of its own accord or induced by artificial means. This layer of dry soil formed at the surface is known as mulch. To this mulch is ascribed the important function of conserving the moisture in the soil by its ability to reduce evaporation of water at the surface. It accomplishes this conservation of moisture, it is claimed, by producing a change or break in the capillary connections between itself and the moist soil below.

Since, on account of the kinetic energy, the absorptive and adhesive forces of the solid substance decrease with a rise in temperature, the interesting question arose whether the dry mulch with an excessively high temperature would absorb moisture from a moist soil with low temperature, even when the capillary connections were ideal. The desire to secure information upon this important and exceedingly interesting point led to the execution of the following experiments: Brass tubes, as described in the preceding sections, were filled with soil, one half with

dry and the other half with moist soil, and the two columns were separated only by a circular piece of cheesecloth, in order to facilitate the separation of the two columns for moisture-movement determinations.

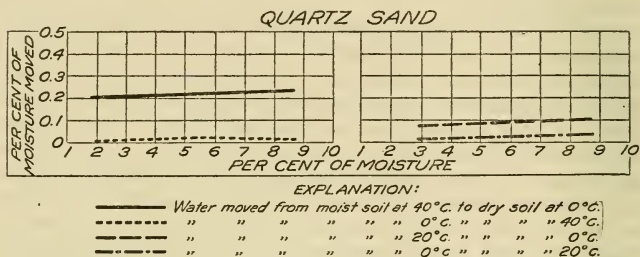


FIG. 5.—Curve showing the percentage of moisture moved from a moist and warm column to a dry and cold column of quartz sand, and from a moist and cold to a dry and warm column of quartz sand.

The tubes were then inserted in the boxes shown in figures 1 and 2, and that portion of the tubes containing the moist soil was kept at 20° and 40°, while that part which held the dry soil was maintained at 0° C.

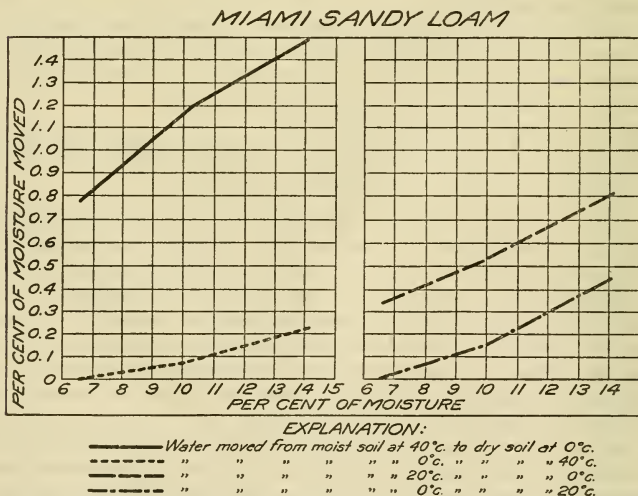


FIG. 6.—Curve showing the percentage of moisture moved from a moist and warm column to a dry and cold column of Miami sandy loam, and from a moist and cold to a dry and warm column of Miami sandy loam.

In another set of tubes these temperatures were reversed. The soils employed were the same as those previously described—namely: Quartz sand, light and heavy Miami sandy loam, Miami silt loam, Clyde silt

loam, and Miami clay. There were three different moisture contents used for each soil, designated as low, medium, and high. The duration of all experiments was about eight hours. The numerical data obtained are shown in Table IV. The accompanying figures 5 to 10 represent these same data graphically. Each soil has two charts: The one to the left is for the temperature amplitude of 40° , and the one to the right is for the temperature range of 20° C. The abscissas in every case represent the percentage of moisture content and the ordinates the percentage of water moved either from the moist and warm column to the dry and cold column of soil, or from the moist and cold column to the dry and warm column of soil. The upper curves of each chart represent the

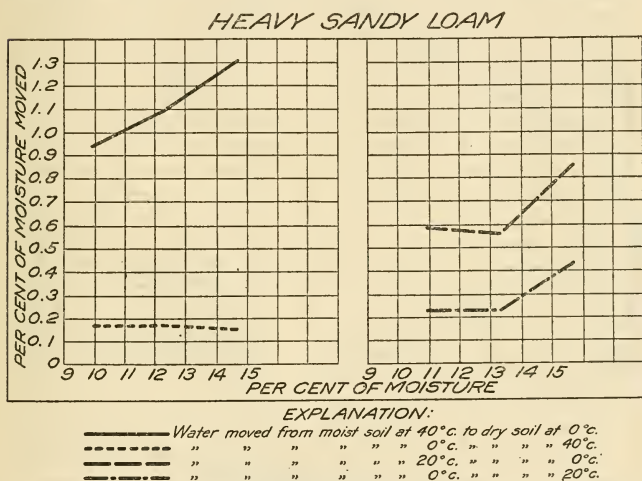


FIG. 7.—Curve showing the percentage of moisture moved from a moist and warm column to a dry and cold column of heavy sandy loam, and from a moist and cold to a dry and warm column of heavy sandy loam.

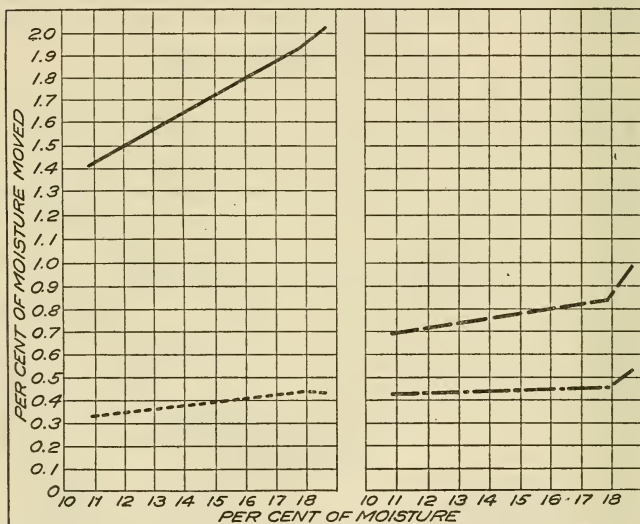
percentage of water movement that took place from the moist and warm soil to the dry and cold soil, while the lower curves show the movement of water that occurred from the moist and cold soil to the dry and warm soil. As in the preceding case, the percentage of moisture moved is based upon the difference in percentages of moisture contained in the dry soil of the beginning and end of the experiment.

Considering first the numerical values showing the amount of water moved from the moist and warm column of soil to the dry and cold column of soil, which are graphically represented by the upper curve of each chart (fig. 5 to 10), it will be seen (1) that this amount is nearly twice as great in the temperature amplitude of 40° as in 20° C., (2) that

it is somewhat greater in soils with higher than with lower colloidal content, and (3) that it increases with the rise in moisture content.

By comparing these results with those obtained with columns of soils of uniform moisture content, some very striking contrasts are revealed. The previous results show, for instance, that the maximum thermal motion of water occurs at a definite but comparatively low moisture content and that the value amounts in some cases to more than 3.50 per cent. The above data show, however, that the maximum movement

MIAMI SILT LOAM



EXPLANATION:

————— Water moved from moist soil at 40°C. to dry soil at 0°C.
 - - - - - " " " " " " " 0°C. " " " " 40°C.
 - - - - - " " " " " " " 20°C. " " " " 0°C.
 - - - - - " " " " " " " 0°C. " " " " 20°C.

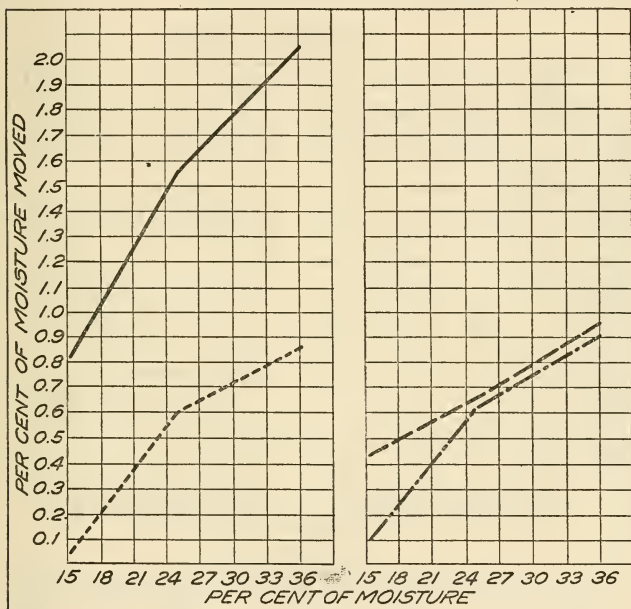
FIG. 8.—Curve showing the percentage of moisture moved from a moist and warm column to a dry and cold column of Miami silt loam, and from a moist and cold to a dry and warm column of Miami silt loam.

of water from the moist and warm column to the dry and cold column of soil takes place at the highest water content and that in the majority of cases the percentage of this maximum water translocation is only one-half as great as in the former case.

These apparent differences seem to be easily explainable. The increase of water movement from moist and warm soil to dry and cold soil with a rise in water content is natural and only goes to prove that the water is held by the soil with low moisture content with great force, and consequently it can not be extracted readily and extensively by a greater

abstracting force. When the attractive forces of the soil for water are satisfied and the thickness of the surface and capillary films is increased, then greater quantities of water will be removed by the same abstracting force. The smaller thermal water movement which occurs in the moist and dry soil rather than in the soil of uniform moisture content is due mainly to the cheesecloth which is placed between the dry and moist

CLYDE SILT LOAM



EXPLANATION:

—	Water moved from moist soil at 40°C. to dry soil at 0°C.
- - -	" " " " " " 0°C. " " " " 40°C.
- - -	" " " " " " 20°C. " " " " 0°C.
- - -	" " " " " " 0°C. " " " " 20°C.

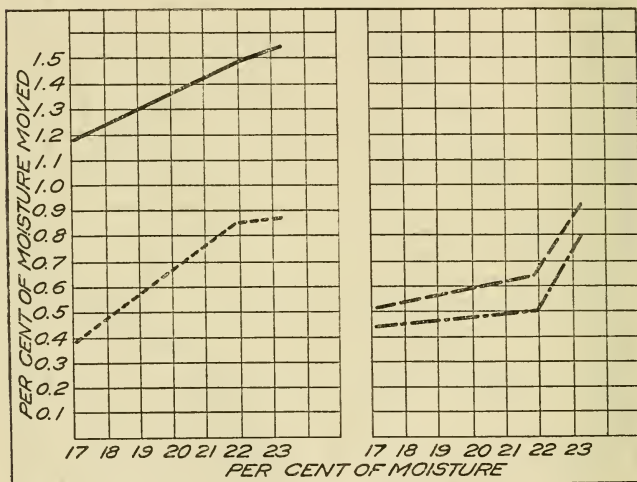
FIG. 9.—Curve showing the percentage of moisture moved from a moist and warm column to a dry and cold column of Clyde silt loam, and from a moist and cold to a dry and warm column of Clyde silt loam.

columns of soil. Although this cheesecloth was very thin and had wide meshes, yet it prevented the two columns from forming a complete and perfect contact; consequently the dry soil had to absorb water directly through the cheesecloth as well as from the soil.

Another factor which would seem to impede the rate of water movement from a moist and warm to a dry and cold column of soil is the resistance which the dry soils offer to wetting, owing to the air film

surrounding the particles and to any oily substances that might be present. The influence of this factor, however, must be extremely small, if any, because when these soils were slightly damped the amount of water moved was generally less or about the same as before. The common belief that water moves more rapidly in damp than in dry soils is generally exaggerated. When a soil is damped to eliminate the factor of resistance to wetting, its absorptive power for water is decreased correspondingly, so that one factor tends to counterbalance the other, and at the end the

MIAMI CLAY



EXPLANATION:

—————	Water moved from moist soil at 40°c. to dry soil at 0°c.
- - - - -	" " " " " " " 0°c. " " " " 40°c.
- · - · -	" " " " " " " 20°c. " " " " 0°c.
- · - · -	" " " " " " " 0°c. " " " " 20°c.

FIG. 10.—Curve showing the percentage of moisture moved from a moist and warm column to a dry and cold column of Miami clay, and from a moist and cold to a dry and warm column of Miami clay.

results are about the same. Moreover, the soils which stubbornly resist wetting are not very common.

From the practical standpoint the results of the second part of the present investigation are probably far more important than those of the first part just discussed. These results show the remarkable fact that when the dry soil is kept at 20° and 40° and the moist soil at 0° C., the dry soil takes up very little, if any, water from the moist soil and that this quantity of water absorbed decreases with a rise in temperature. As will be seen from the data, the percentage of moisture absorbed by the dry soil at 20° is in all cases greater than that absorbed at 40° C.

At both amplitudes of temperature the percentage taken up increases with the colloidal content in the soil, which is natural.

TABLE IV.—*Movement of moisture from a moist and warm column of soil to a dry and cold column of soil and from a moist and cold column of soil to a dry and warm column of soil*

Kind and temperature of soil.		Percentage of moisture in soil.		
Quartz sand:				
At beginning of experiment.....	1. 85	5. 30	8. 75	
Movement from moist column at 20° to dry column at 0° C.....	. 0746	. 0879	. 1129	
Movement from moist column at 0° to dry column at 20° C.....	. 0105	. 02131	. 03912	
Movement from moist column at 40° to dry column at 0° C.....	. 2048	. 2210	. 2376	
Movement from moist column at 0° to dry column at 40° C.....	. 0121	. 0160	. 01522	
Light sandy loam:				
At beginning of experiment.....	6. 497	10. 141	14. 17	
Movement from moist column at 20° to dry column at 0° C.....	. 345	. 550	. 820	
Movement from moist column at 0° to dry column at 20° C.....	. 061	. 163	. 448	
Movement from moist column at 40° to dry column at 0° C.....	. 779	1. 18	1. 496	
Movement from moist column at 0° to dry column at 40° C.....	. 000	. 08	. 235	
Heavy sandy loam:				
At beginning of experiment.....	9. 906	12. 30	14. 695	
Movement from moist column at 20° to dry column at 0° C.....	. 592	. 569	. 863	
Movement from moist column at 0° to dry column at 20° C.....	. 215	. 211	. 445	
Movement from moist column at 40° to dry column at 0° C.....	. 937	1. 094	1. 309	
Movement from moist column at 0° to dry column at 40° C.....	. 168	. 169	. 150	
Silt loam:				
At beginning of experiment.....	10. 89	17. 88	18. 67	
Movement from moist column at 20° to dry column at 0° C.....	. 687	. 844	. 989	
Movement from moist column at 0° to dry column at 20° C.....	. 411	. 461	. 529	
Movement from moist column at 40° to dry column at 0° C.....	1. 413	1. 942	2. 038	
Movement from moist column at 0° to dry column at 40° C.....	. 347	. 445	. 438	
Clyde silt loam:				
At beginning of experiment.....	15. 349	25. 086	36. 18	
Movement from moist column at 20° to dry column at 0° C.....	. 429	. 662	. 962	
Movement from moist column at 0° to dry column at 20° C.....	. 100	. 606	. 900	
Movement from moist column at 40° to dry column at 0° C.....	. 814	1. 554	2. 046	
Movement from moist column at 0° to dry column at 40° C.....	. 042	. 594	. 852	

TABLE IV.—*Movement of moisture from a moist and warm column of soil to a dry and cold column of soil and from a moist and cold column of soil to a dry and warm column of soil—Continued*

Kind and temperature of soil.	Percentage of moisture in soil.		
Clay:			
At beginning of experiment.....	17.05	21.88	23.29
Movement from moist column at 20° to dry column at 0° C.....	.514	.653	.923
Movement from moist column at 0° to dry column at 20° C.....	.436	.502	.796
Movement from moist column at 40° to dry column at 0° C.....	1.180	1.482	1.552
Movement from moist column at 0° to dry column at 40° C.....	.380	.850	.873

Obviously, then, the temperature has a tremendous influence upon the absorptive power of soils for water. This is what might be expected from the laws of kinetic energy. According to this law, the energy or motion of the molecules increases with temperature, and consequently the adhesive and absorptive forces of the solid matter for liquids or gases decreases. These results, then, tend to confirm postulate 2 (p. 148), that the attractive forces of the soil for water decrease with a rise in temperature.

The foregoing experimental results and theoretical considerations suggest very strongly that the efficiency of the soil mulches in conserving moisture in the soil is not dependent solely upon their thickness and degree of capillary discontinuity between themselves and the moist soil below, but also upon their temperature. It is well known that the temperature of the surface soils during sun insolation is many degrees higher than that of the air immediately above. In some parts of the world where the sky is clear and the sun insolation very intense, the surface soil may attain a temperature about 40° C. higher than that of the air about 4 feet from the ground. Even at this Station the surface soil temperature of the mineral soils, and especially of the light sandy soils, is very often approximately 15° C. higher than that of the air above. From the surface downward the soil temperature decreases, but in the upper 1 or 2 inches the diminution is far more rapid than at the lower depths, amounting sometimes and in certain soils to more than 11° C. for each inch in depth. When the surface soil is disturbed and a mulch is formed, its heat conductivity is decreased, and the high temperature attained at the surface is not all conducted downward but is compelled to accumulate on the dry mulch and then is radiated back into space. The difference in temperature between the mulch and the moist soil below is sometimes as high as 15° C. at this Station. In arid regions this difference must be far greater.

This excessively greater temperature of the dry mulch diminishes the adhesive and absorptive forces of the dry soil, so that its capacity and intensity to withdraw water from the moist soil below are either entirely prohibited or greatly reduced. The result is that the water is saved from direct evaporation. On the other hand, during the night the soil temperature reverses itself and becomes lowest at the surface and increases with the depth, but the difference between the mulch and moist soil is generally not as great during night as during the sun insolation. Since the attractive and adhesive force of the dry soil and the surface tension of the soil water are increased by the low temperature, the tendency of the soil moisture is to move upward very energetically. To what extent this movement occurs can not be stated with certainty, because the moisture not very far below the mulch is held with a great force and is given up with great reluctancy unless moisture moves from a farther depth below, satisfies the absorptive power, and thickens the surface and capillary films.

Furthermore, the amount of water moved will depend upon the temperature gradient—that is, upon the range of temperature between the surface and lower depths. As already stated, this temperature gradient at night is most marked during the summer and fall and is smallest during the spring. Any water, however, that the mulch pulls up during the night is certain of being evaporated during the day. May it not be, then, that an appreciable amount of water is lost from the soil in this manner?

Temperature not only tends to conserve moisture in the soil after the mulch is formed but also aids and hastens the formation of this mulch. It has been seen that as the temperature of the moist soil at the upper depth increases, the surface tension of the soil water and the adhesive and absorptive forces of the soil decrease. The upward pulling force, therefore, is diminished, and the water is not brought up with sufficient rapidity to keep the upper layers moist, so that a mulch is formed at the top. The diminution of the surface tension of the soil water at or near the surface is very large during the sun insolation and far greater than the increase during the night, because during the sun insolation the soil absorbs heat from the sun very rapidly, and since the soil is a poor conductor of heat the heat is allowed to accumulate at the surface and raise its temperature far above that of the next layers.

The foregoing considerations have been deduced from the experimental data and from the laws of kinetic energy of matter, surface tension of liquids, etc., in their relation to temperature. It is now of great importance as well as of high interest to know whether these deductions can be verified experimentally. The type of experiment which the writer probably would have performed to test out whether or not the temperature does tend to conserve moisture in the soil has

fortunately been performed by Buckingham (5) for another purpose. In his studies on the loss of water under arid and humid conditions, Buckingham endeavored to imitate these two conditions in the laboratory. He placed soil in cylinders 48 inches long and $2\frac{1}{2}$ inches in diameter and provided each cylinder with side tubes at the bottom for the introduction of water. By means of an electric fan he allowed a current of air to be blown over the top surface of the soils. For the arid conditions this current of air was heated without changing its absolute humidity to a temperature of about 50° to 60° F. above the room temperature. To imitate also the high surface temperature of soils under the strong sunshine of arid climates, the top $1\frac{1}{2}$ inches of the cylinders under the hot air was heated by coils surrounding the cylinders to about the same temperature as the hot air. The breeze of about 3 miles per hour was kept going all the time. The heating current was turned on for six

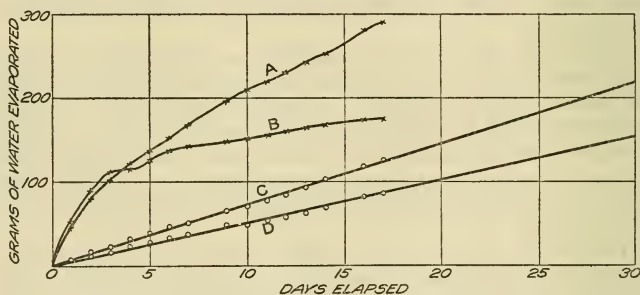


FIG. 11.—Curve showing the evaporation of water from Takoma soil fed with tap water: A, Soil under humid conditions; B, soil under arid conditions; C, water under arid conditions; D, water under humid conditions.

hours a day, except on Sundays and holidays. For the humid conditions the soils were placed under the current of air at room temperature. Buckingham performed a number of experiments bearing upon this subject and the results he obtained are qualitatively about the same for all of them. Figure 11 shows a typical set of results.

An examination of figure 11 shows that the loss of water from the soil under arid conditions is much more rapid at first, but after about 4 days have elapsed the rate of loss is less under arid than under humid conditions and continues to be so throughout the duration of the experiment. The rate of evaporation from the soils for the last 10 days is 11.2 inches of rain per year under arid conditions and 51.6 inches of rain per year under humid conditions.

Buckingham explains these results under the supposition that a mulch was formed on the soil kept under arid conditions more rapidly than on the soil kept under humid conditions, and the mulch prevented rapid loss

of water from the former. This explanation is correct, of course, in so far as it represents the result of the mulch, but how this mulch was formed and how it was capable of accomplishing this result he fails to explain correctly. In the opinion of the writer the above results offer an excellent proof that temperature aids and hastens the formation of a mulch, and tends to conserve the soil moisture in the manner previously set forth.

This is a remarkable paradox indeed that a temperature which causes the loss of water should also cause its conservation.

SUMMARY

The main and most important facts presented in the foregoing series of studies may be summarized as follows:

(1) When one half of a column of soil of uniform moisture content is maintained at 20° and 40° and the other half at 0° C. for eight hours the percentage of water moved from the warm to the cold soil increased in all the different types of soil with a rise in moisture content until a certain water content was reached, and then it began to decrease again with further increase in moisture content. The results then plot into a parabola. The percentage of moisture at which the maximum thermal translocation of water occurred is different for the diverse classes of soil, but the percentage of the maximum thermal translocation of water is about the same for all classes of soil for any one of the temperature amplitudes. The percentage of moisture at which this maximum thermal translocation occurred is designated as the "thermal critical moisture content."

These results are contrary to what might be expected from the laws of surface tension and viscosity. They have led to the conclusion that the capillary movement of water in moist soils is not controlled entirely by the curvature of the capillary films, as is generally believed, but also by the unsatisfied attractive forces of the soil for water.

(2) When a moist column of soil was kept at 20° and 40° and a dry column of soil at 0° C. for eight hours and the two columns were separated by an air space, the percentage of moisture distilled over from the moist and warm column to the dry and cold column of soil was very insignificant for both amplitudes of temperature and was about the same for all moisture contents.

These results lead to the conclusion (a) that the amount of water lost from the soil by water vapor is very small; (b) that there is no rising of vapor during the night from the warmer soil below to the cold soil above; and (c) that the water of the dew is not derived from the soil vapor, as is commonly believed.

(3) When a moist column of soil was in contact with a dry column of soil and the former was kept at 20° and 40° and the latter at 0° C. for eight hours the amount of moisture moved from the moist and warm soil

to the dry and cold soil increased with temperature and with moisture content. But when the moist column of soil was maintained at 0° and the dry column of soil at 20° and 40° C. for the same number of hours there was very little, if any, movement of water from the former to the latter.

These results have led to the conclusion that temperature has a very marked influence on the conservation of moisture by mulches.

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SOIL TEMPERATURES AS INFLUENCED BY CULTURAL METHODS

By JOSEPH OSKAMP,

Research Assistant in Pomology, Purdue University Agricultural Experiment Station

The data here reported were accumulated under natural field conditions during a period of two years on three plots in a young apple orchard, as follows: (1) Tillage with a cover crop, (2) straw mulch, and (3) grass land. The temperature effect of cultural methods is a detail of a general investigation of the phenomena of orchard soil management. The data have a bearing on other soil problems perhaps important enough to warrant separate presentation at this time.

The temperatures were recorded automatically by means of soil thermographs manufactured by Julien P. Friez & Sons. This instrument consists of a cylinder revolved by an 8-day clock. Blank forms are placed on the cylinder and the temperatures are traced thereon by a pen connected with the thermometer bulb. Temperatures are thus recorded continuously.

The thermometer bulbs were planted 5 or 6 feet northeast of each tree and at a depth of 9 inches. On the straw-mulch plot the bulb was placed under and 12 inches from the outer edge of the mulch collar. Only one instrument was used on each plot. It is felt, however, that the records are trustworthy and portray with reasonable exactness the existing conditions. All instruments were carefully checked with a standard thermometer at the beginning and during the course of the experiment, and their behavior was highly satisfactory. Great care was exercised in changing the chart sheets, to see that each blank was properly placed.

The plots are located on a glaciated, rough, river-bluff, upland soil in southern Indiana. The rocks of the region are limestone, which out-crop on the steeper hillsides. The mechanical analysis shows the soil to be a clay silt. (See Table I.)

TABLE I.—*Mechanical analysis of upper 9 inches of soil on the experimental plots ^a*

Plot.	Fine gravel (.2 to 1 mm.).	Coarse sand (1 to 0.5 mm.).	Medium (0.5 to 0.25 mm.).	Fine sand (0.25 to 0.1 mm.).	Very fine sand (0.1 to 0.05 mm.).	Silt (0.05 to 0.005 mm.).	Clay (0.005 to 0 mm.).
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
A.....	0.1	0.7	0.8	1.3	1.8	82.1	13.0
C.....	.2	.8	.9	1.4	5.2	78.7	12.5
D.....	.2	.7	.8	1.6	7.3	77.0	12.4

^a These analyses were made by the Bureau of Soils, United States Department of Agriculture.

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Plot A received clean cultivation with a rye cover crop sown in late summer and turned under in the spring. The average depth of plowing has been 7 inches. Cultivation started in 1913 on May 1 and in 1914 on May 11. Rye at the rate of $1\frac{1}{2}$ bushels per acre was sown for a cover crop on August 20 in 1913 and on August 15 in 1914. The land was cultivated seven times each season.

Plot C was in grass, which was cut and allowed to lie where it fell, as in plot D, but in addition a mulch of wheat straw was applied about the same time that plot A was plowed, using one bale to a tree. The bales averaged 93 pounds in weight. The litter was scattered around the trees, forming a collar 12 to 14 feet in diameter.

Plot D was in grass, which was cut and allowed to lie where it fell. In the autumn of 1912 plot D was seeded to a mixture of grasses in which timothy largely predominated and may here be considered as a timothy meadow. The grass was mowed for the first time in the middle of June, 1913, largely to prevent weeds from seeding, as the amount of mulch was negligible. The extremely dry summer of 1914 was disastrous to grass, and a cutting on July 10 returned to the land only one-fifth of a ton of dry hay per acre.

Space does not permit the publication of the complete temperature records, but the weekly maximums and minimums are given in Table II. It will be seen that in April plot A began to forge ahead in holding the highest minimum temperature, with plot D second and C third. This condition prevailed until in the fall, when plot A cooled off quickly and D less quickly, leaving C with the highest minimum temperature until spring. The differences, however, in winter temperatures between the plots were small. During the week of February 23, 1913, plot A showed a minimum temperature of 32° F. and plots C and D, 32.5° F. Plot A continued to cool, until during the week of March 16 it reached 32° , when plot D registered its lowest, 31° , and plot C was 33° F. The following winter the three plots reached their minimum temperatures during the week of January 11, that of plot A being 31° ; D, 32.5° ; and C, 34° F.

TABLE II.—Records of soil temperatures under different cultural methods, May, 1913, to May, 1915

PLOT A: TILLAGE WITH COVER CROP

Week ending—	Mini-mum.	Maxi-mum.	Week ending—	Mini-mum.	Maxi-mum.	Week ending—	Mini-mum.	Maxi-mum.
	$^{\circ}$ F.	$^{\circ}$ F.		$^{\circ}$ F.	$^{\circ}$ F.		$^{\circ}$ F.	$^{\circ}$ F.
May 5	45.0	58.5	June 16	58.5	68.0	July 28	67.0	76.0
12	53.0	60.0	23	67.0	74.0	Aug. 4	72.0	80.0
19	53.0	63.0	30	67.5	78.0	11	70.0	77.5
26	54.0	63.0	July 7	73.0	80.5	18	71.0	78.0
June 2	54.5	66.5	14	69.0	77.5	25	66.0	77.0
9	60.0	69.0	21	69.0	77.5	Sept. 1	66.0	73.0

TABLE II.—Records of soil temperatures under different cultural methods, May, 1913, to May, 1915—Continued

PLOT A: TILLAGE WITH COVER CROP—continued

Week ending—	Mini- mum.	Maxi- mum.	Week ending—	Mini- mum.	Maxi- mum.	Week ending—	Mini- mum.	Maxi- mum.
	^{°F.}	^{°F.}		^{°F.}	^{°F.}		^{°F.}	^{°F.}
Sept. 8	68.0	76.5	Mar. 30	33.0	50.0	Oct. 19	56.0	60.0
15	59.0	75.0	Apr. 6	41.0	51.0	26	53.0	60.0
22	55.5	66.0	13	38.0	46.0	Nov. 2	45.0	57.5
29	51.0	61.0	20	42.5	55.5	9	45.0	53.0
Oct. 6	55.0	63.0	27	44.0	61.5	16	43.0	52.0
13	54.0	65.0	May 4	51.5	66.0	23	34.0	46.0
20	50.0	59.0	11	52.5	63.0	30	33.0	45.0
27	43.5	51.0	18	52.5	64.0	Dec. 7	43.0	50.5
Nov. 3	25	56.0	67.0	14	37.0	43.0
10	41.0	48.0	June 1	64.0	74.0	21	34.5	37.0
17	37.0	48.5	8	65.5	73.5	28	34.0	35.0
24	45.0	56.0	15	70.0	77.0	Jan. 4	32.0	33.5
Dec. 1	43.0	52.5	22	67.0	74.0	11	31.0	32.0
8	43.0	53.0	29	68.0	79.0	18	31.0	32.0
15	36.0	42.0	July 6	65.0	75.5	25	32.0	33.0
22	36.0	41.0	13	70.0	80.0	Feb. 1	33.0	34.0
29	36.0	37.0	20	68.0	78.0	8	33.0	36.0
Jan. 5	35.0	36.0	27	70.0	80.0	15	32.0	45.0
12	34.0	35.0	Aug. 3	71.0	79.0	22	35.0	40.0
19	33.0	34.0	10	72.0	78.0	Mar. 1	35.0	47.0
26	33.0	37.0	17	70.0	77.5	8	34.0	37.0
Feb. 2	34.0	46.5	24	70.0	80.0	15	35.0	41.0
9	34.0	40.0	31	66.5	74.5	22	36.0	40.0
16	33.0	34.0	Sept. 7	64.0	74.5	29	35.0	41.0
23	32.0	33.0	14	59.5	72.0	Apr. 5	35.0	42.0
Mar. 2	31.0	33.0	21	61.5	73.5	12	40.0	52.5
9	31.0	32.0	28	57.0	74.0	19	44.0	55.0
16	30.0	32.5	Oct. 5	58.0	64.5	26	50.0	66.0
23	31.0	34.0	12	59.0	67.5	May 3	57.0	60.0

PLOT C: STRAW MULCH

May 5	47.0	53.0	Oct. 6	58.0	61.0	Mar. 9	33.0	34.0
12	50.0	53.0	13	57.0	61.5	16	33.0	33.5
19	50.0	56.0	20	51.5	58.5	23	33.0	34.0
26	55.0	57.0	27	50.0	54.0	30	33.0	37.0
June 2	56.0	60.0	Nov. 3	Apr. 6	37.5	42.0
9	58.5	61.5	10	45.0	48.0	13	38.0	43.0
16	57.0	60.0	17	42.0	47.0	20	40.0	48.0
23	60.0	65.0	24	46.0	51.5	27	44.5	49.5
30	64.0	70.0	Dec. 1	47.0	51.0	May 4	49.0	51.5
July 7	68.0	71.0	8	46.5	51.0	11	51.0	52.5
14	67.0	69.0	15	41.0	47.0	18	51.0	54.5
21	68.0	71.0	22	39.5	42.5	25	52.0	55.0
28	66.0	68.5	29	38.0	40.0	June 1	56.0	59.5
Aug. 4	68.0	70.0	Jan. 5	37.5	38.5	8	58.0	62.0
11	66.0	70.0	12	36.0	39.0	15	63.0	65.0
18	70.0	72.0	19	34.0	36.0	22	60.5	64.0
25	68.0	72.0	26	36.0	38.0	29	64.0	67.0
Sept. 1	66.0	69.0	Feb. 2	37.0	41.5	July 6	63.0	65.0
8	66.0	70.0	9	35.0	39.0	13	64.0	67.5
15	62.0	69.0	16	34.0	35.0	20	66.0	68.0
22	61.0	64.5	23	32.5	35.0	27	66.0	69.0
29	57.5	61.0	Mar. 2	33.0	34.0	Aug. 3	65.0	70.0

TABLE II.—Records of soil temperatures under different cultural methods, May, 1913, to May, 1915—Continued

PLOT C: STRAW MULCH—continued

Week ending—	Mini- mum.	Maxi- mum.	Week ending—	Mini- mum.	Maxi- mum.	Week ending—	Mini- mum.	Maxi- mum.
	°F.	°F.		°F.	°F.		°F.	°F.
Aug. 10	65.0	67.5	Nov. 9	51.0	52.5	Feb. 8	35.0	37.0
17	65.0	68.5	16	48.5	52.0	15	35.0	39.0
24	66.0	68.5	23	40.0	50.0	22	36.0	38.5
31	65.0	68.0	30	41.0	45.0	Mar. 1	36.0	40.0
Sept. 7	64.0	67.5	Dec. 7	45.5	47.5	8	36.0	37.0
14	61.5	66.0	14	43.0	46.0	15	36.0	37.0
21	61.5	64.0	21	38.0	43.0	22	37.0	38.0
28	59.0	65.0	28	36.0	38.5	29	36.5	38.0
Oct. 5	58.0	60.0	Jan. 4	35.0	37.0	Apr. 5	35.5	37.0
12	60.0	62.5	11	34.0	36.0	12	38.0	45.0
19	58.0	60.0	18	35.0	36.0	19	42.0	45.0
26	51.0	56.0	25	35.0	36.5	26	45.0	53.0
Nov. 2	56.0	58.0	Feb. 1	35.0	36.0	May 3	52.0	55.0

PLOT D: GRASS LAND

May 5	44.5	57.5	Jan. 5	36.0	37.0	Sept. 7	63.0	70.0
12	48.5	59.0	12	35.5	37.0	14	60.0	67.5
19	49.0	62.0	19	33.5	36.5	21	60.0	66.0
26	53.0	62.5	26	33.0	36.5	28	56.0	66.5
June 2	53.5	66.0	Feb. 2	35.0	44.0	Oct. 5	55.0	60.0
9	58.0	67.0	9	35.0	39.5	12	58.0	62.5
16	57.0	69.0	16	34.0	36.0	19	56.0	58.0
23	66.0	74.0	23	32.5	35.0	26	54.0	56.5
30	65.0	76.5	Mar. 2	32.0	35.0	Nov. 2	46.0	53.0
July 7	70.0	78.5	9	32.0	34.0	9	47.0	51.0
14	67.5	76.0	16	31.0	34.0	16	44.0	50.0
21	23	33.0	34.0	23	37.0	47.0
28	30	32.0	46.0	30	35.0	44.0
Aug. 4	69.0	76.0	Apr. 6	40.0	48.0	Dec. 7	44.0	47.5
11	67.0	74.5	13	37.5	44.0	14	40.0	44.0
18	68.0	74.0	20	41.0	53.0	21	35.5	41.0
25	64.0	73.5	27	44.0	57.0	28	34.5	36.5
Sept. 1	62.5	68.5	May 4	50.5	61.0	Jan. 4	35.0	37.0
8	64.0	69.5	11	52.0	58.5	11	32.5	35.0
15	57.5	69.0	18	53.0	60.5	18	33.0	34.0
22	55.5	62.5	25	54.0	62.0	25	33.5	35.5
29	51.0	57.0	June 1	60.5	67.0	Feb. 1	33.0	35.0
Oct. 6	52.5	59.5	8	60.5	69.0	8	33.0	35.0
13	52.0	60.0	15	65.0	71.0	15	32.5	38.5
20	49.0	56.0	22	63.5	70.0	22	33.0	38.0
27	43.0	47.0	29	67.5	73.5	Mar. 1	35.0	41.0
Nov. 10	41.5	47.0	June 6	63.5	72.0	8	33.0	35.0
17	39.0	49.0	13	68.0	76.0	15	33.5	36.5
24	45.0	54.0	20	67.0	75.0	22	35.0	37.0
Dec. 1	43.5	51.5	27	68.0	77.0	29	35.0	38.0
8	44.0	52.0	Aug. 3	68.0	74.0	Apr. 5	34.0	38.0
15	36.5	44.5	10	69.0	74.0	12	37.0	47.5
22	36.5	41.0	17	67.0	73.5	19	42.5	48.0
29	36.5	37.0	24	68.0	75.5	26	46.0	58.0
			31	65.0	71.0	May 3	53.0	59.0

Plot A maintained the highest maximum temperature during the spring and summer and until quite cold weather in the fall, when plot C registered the highest maximum temperature. This lasted for a month or so during the coldest weather, and as soon as it began to moderate in late winter plot A warmed up rapidly, with D next.

The greatest variation between plots occurred during the summer months. In the spring and fall there is a transition period in which the temperature differences are less. During the summer of 1913 plot A registered a maximum temperature of 80.5° the week of July 7, when plot D was 78.5°, and plot C was 71° F. However, plot C later, the week of August 18, registered a maximum temperature of 72° F. During the week of July 13, the following summer, plot A registered a maximum of 80°; plot D, 77°, the week of July 24; and plot C, 70° F., the week of August 3.

Figures 1 to 4 are reproductions of typical seasonal charts of soil temperatures prevailing under the three cultural systems. These give an idea of the diurnal variations. During the winter the temperatures are quite constant from day to day, with very little variation between plots (fig. 1). In the spring the diurnal range is considerable in the plot under tillage with cover crop and the grass land, but varies little under the straw mulch, which exhibits a very gradual warming up (fig. 2). During the summer season, fluctuations become quite pronounced under tillage and grass, but the straw mulch still maintains its uniformity (fig. 3, C). During the season of greatest daily range the maximum and minimum temperatures occur about 10 p. m. and 10 a. m., respectively (fig. 3, A and D). In the fall the temperatures and ranges are not radically different from those of spring, except that the general trend of temperatures is reversed (fig. 4).

In conclusion, it may be said that a system of clean cultivation with a winter cover crop is characterized by extreme diurnal and annual fluctuations in soil temperature; that a straw mulch equalized these fluctuations to a marked extent, as does also a grass crop, though in less degree.

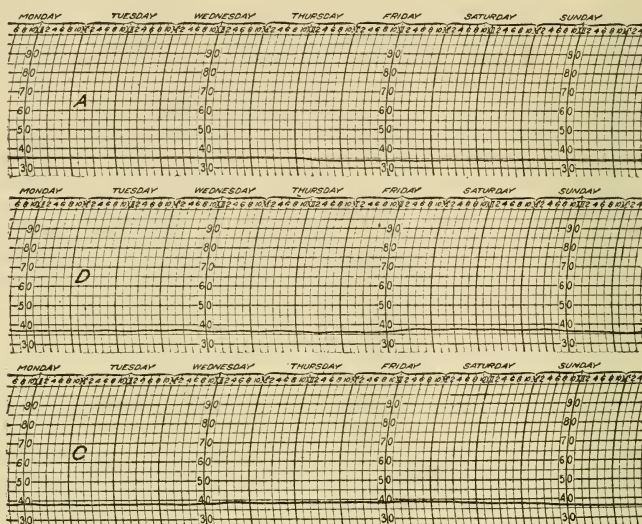


FIG. 1.—Typical charts of soil temperatures during the winter season: Records for week ending January 12, 1914. A, Tillage with cover crop; D, grass land; C, straw mulch.

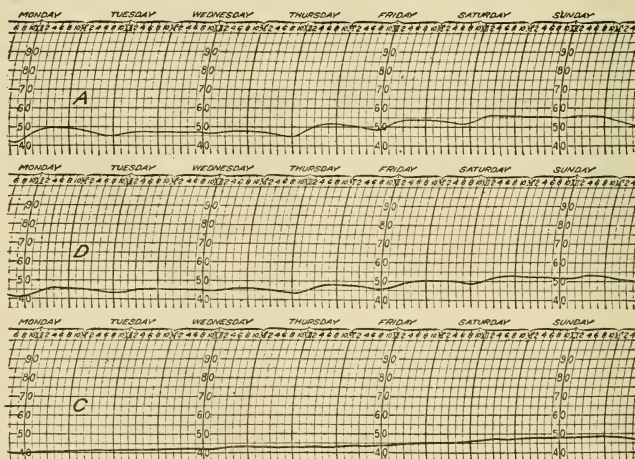


FIG. 2.—Typical charts of soil temperatures during the spring time: Records for week ending April 20, 1914. A, Tillage; D, grass land; C, straw mulch.

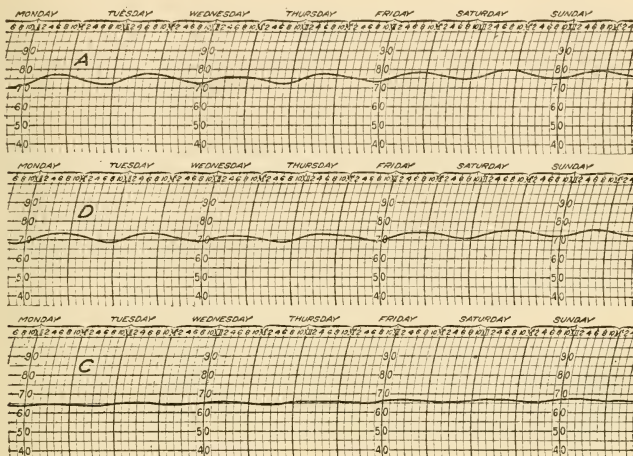


FIG. 3.—Typical charts of soil temperatures during the summer months: Records for week ending June 13, 1914. A, Tillage; D, grass land; C, straw mulch.

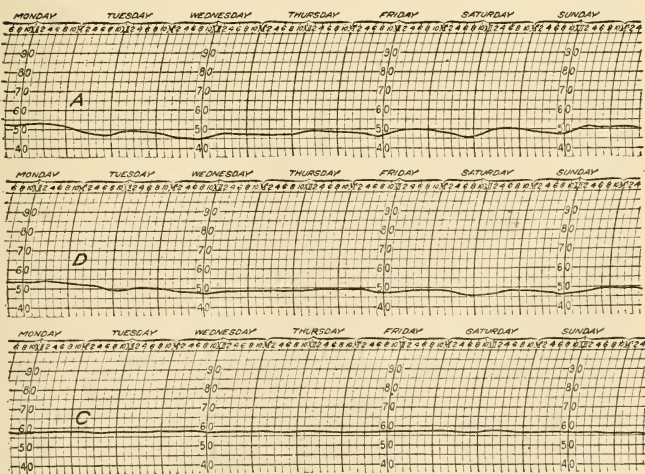


FIG. 4.—Typical charts of soil temperatures during the fall of the year. Records for week ending November 2, 1914. A, Tillage with cover crop; D, grass land; C, straw mulch.



ALTERNARIA PANAX, THE CAUSE OF A ROOT-ROT OF GINSENG

By J. ROSENBAUM, *Specialist in Phytophthora*, and C. L. ZINNSMEISTER, *formerly Agent, Cotton and Truck Disease Investigations, Bureau of Plant Industry*

While working with diseases of ginseng (*Panax quinquefolium*) during the summer of 1913, the authors obtained from a garden near Cleveland, Ohio, roots which showed a peculiar dry-rotted condition about the crown. The dark-brown center of the lesion characterizing this dry-rot was more or less sunken and firm to the touch and gradually shaded into the yellowish white color of the healthy root. It is distinguished from other root-rots by its lack of odor and the fact that the rotted roots never become soft. Plate XII is a reproduction of a photograph of three roots showing the typical lesions of the disease.

When the rot is near the crown of the root, the top of the plant often shows signs of the disease. These signs are a wilting and yellowing of the leaves, which on being disturbed drop off readily at the point of attachment to the main stalk. Such a condition may, however, be caused by other root-rots attacking ginseng, as, for example, the rot caused by *Phytophthora cactorum*.

Because of the unusual character of these lesions, numerous isolations were made from them, and in all cases an *Alternaria*-like fungus closely resembling *Alternaria panax* Whet. was secured in pure culture. In order to determine whether these two fungi were identical, a series of inoculations on roots and tops were made with both cultures. In addition, a study was made of their macroscopic and microscopic appearance. This work was begun during the summer of 1913 in Ohio and repeated during the summer of 1914 in New York.

In the main two methods of inoculation were followed. Healthy roots were taken from the garden, washed, freed from their fiber roots, sterilized for 10 minutes in a 1 to 1,000 solution of mercuric chlorid, washed in sterile distilled water, and placed in sterilized test tubes. The roots were then injured by making an incision in them with a sterile scalpel, and in this incision was placed a small portion of the fungus from a pure culture. Roots treated in the same way but not inoculated were used as checks. Six series of inoculations were made in this manner, using the *Alternaria*-like fungus isolated from dry-rotted roots. Ninety-five per cent of infection was secured, and the checks in all cases remained healthy. Typical lesions (Pl. XII) were produced in every instance. In no case did the rotted condition involve the entire root. The time necessary after inoculation for the lesion to appear varied from seven to nine days. Once established the progress of the rot was also very slow.

At the time the above series were being run, five series of similar inoculations were made with a pure culture of *Alternaria panax*, the necessary checks for each series being used. One hundred per cent of infection was obtained with this fungus, the symptoms and lesions resulting from the inoculation being in every case similar to and indistinguishable from those obtained with the *Alternaria*-like fungus. Plate XIII, figure 1, shows a longitudinal section through one inoculated root.

In order to test further the pathogenicity of these fungi and to confirm their identity, inoculations were made directly in the soil on roots to which the tops were still attached. Six series were made with the *Alternaria*-like fungus and five with *Alternaria panax*. The soil was removed from around the crown of the roots and an incision was made in the crown. Into this incision was placed the inoculating material from pure cultures of the two fungi. Ninety-two per cent of infection resulted from the *Alternaria*-like fungus and eighty-five per cent from *Alternaria panax*. The symptoms and lesions were again characteristic and similar in each case.

Further inoculations were made on the tops by inoculating the leaves with mycelium from pure cultures of both fungi. For some unexplainable reason, or owing to the plants having been sprayed with Bordeaux mixture, no definite results were secured during the summer of 1913. In June, 1914, the work was repeated. Typical leaf-spots of *Alternaria panax* were produced in abundance with both fungi. Plate XIII, figure 2, is a reproduction of a photograph of the lesions produced on ginseng leaves with the species of *Alternaria* isolated from roots. Spores from these spots were secured and examined. No differences could be noted.

Reisolations were made from the inoculated roots and leaves, and a fungus identical with the original one used for inoculating was obtained.

Numerous attempts to produce infection on the roots without previously injuring them gave only negative results.

Inasmuch as these fungi show no cultural differences and as both are able to infect the leaves and roots of the ginseng plant, the only conclusion warranted by the data at our disposal is that they are identical. This being the case, the blight problem confronting the ginseng grower becomes more complicated. Heretofore it has not been supposed that *Alternaria panax* is able to cause a rot of the root.

The above facts warrant the ginseng grower in taking other means besides spraying in the control of this disease. The means recommended, in addition to spraying, are (1) care in transplanting so as to injure the roots as little as possible, (2) the removal of all tops and stems in the fall, and (3) where the crowns of the roots are sufficiently deep below the surface of the soil, burning over the surface of the bed with a thin layer of straw after the tops have been removed.

PLATE XII

Lesions on ginseng roots due to *Alternaria panax*.



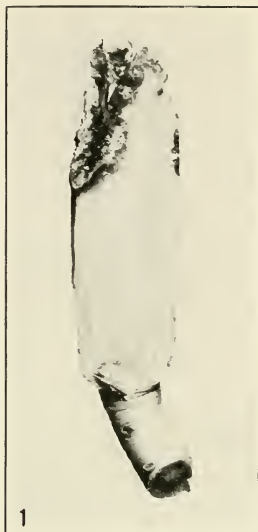


PLATE XIII

Fig. 1.—Longitudinal section of ginseng root showing the results of inoculation with *Alternaria panax*.

Fig. 2.—Inoculations on ginseng leaves with the species of *Alternaria* isolated from ginseng roots.

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SOME POTATO TUBER-ROTS CAUSED BY SPECIES OF FUSARIUM

By C. W. CARPENTER,¹

Scientific Assistant, Cotton and Truck Disease Investigations,
Bureau of Plant Industry

INTRODUCTION

Deterioration of tubers of the Irish potato (*Solanum tuberosum*) is induced by a variety of causes. Economically the most important of these are the organisms *Phytophthora infestans*, *Fusarium* spp., bacteria, and miscellaneous fungi, including *Rhizopus nigricans*.

Phytophthora infestans, which is somewhat restricted to the northeastern part of the country, does more or less damage each year, and occasionally in epidemic form causes tremendous losses. Exclusive of *P. infestans*, however, species of *Fusarium* are undoubtedly the most important causes of tuber decay. Though never occurring in epidemic form with losses comparable to those of late-blight, they are present wherever potatoes are grown, taking their quota of the crop both in the field and in storage.

Several species of the genus *Fusarium* Link have been described as causes of tuber-rots of *Solanum tuberosum* (Clinton, 3; Pizzigoni, 12; Wehmer, 15; Smith and Swingle, 14; Pethybridge and Bowers, 11; Longman, 6; Manns, 7).² In most cases prior to 1912 *F. solani* (Mart.) Sacc. or some species thought to be a synonym of it is given as the causal organism. Until recently the chaotic condition of the genus *Fusarium* has precluded careful work with clearly defined species.

¹ Having been associated with Dr. H. W. Wollenweber, of the Bureau of Plant Industry, during the past two years, the writer has enjoyed the privilege of personal work with the species and strains cultivated during this period in connection with his monographic study of the genus *Fusarium*. Any attempt to work with the species of this form genus emphasizes the necessity of completing such studies. Owing to Dr. Wollenweber's absence during the preparation and publication of this paper, he is not responsible for the subject matter. It is regretted that his criticism of the results is lacking, particularly as the data obtained force the author to conclusions which differ somewhat from Dr. Wollenweber's published opinions.

² Reference is made by number to "Literature cited," pp. 208-209.

For a list of the more important references to potato studies, see the following: Appel, Otto. Beiträge zur Kenntnis der Kartoffelpflanze und ihre Krankheiten. I. In Arb. K. Biol. Anst. Land u. Forstw., Bd. 5, Heft 7, p. 415-435. 1907.

Conclusive work on species of *Fusarium* which produce tuber-rot with sufficiently delimited species dates from Appel and Wollenweber's fundamental work on the form genus *Fusarium*. During the progress of these studies Wollenweber established the wound parasitic nature of *Fusarium coeruleum* (Lib.) Sacc. and *F. discolor*, var. *sulphureum* (Schlecht.) App. and Wollenw., and the causal relation of these species to a definite type of rot. Jamieson and Wollenweber in 1912 (5) described an external dry-rot caused by *F. trichothecioides* Wollenw. Wollenweber in 1913 (19, 20) extended the list of species of *Fusarium* causing tuber-rot by the addition of the following: *F. ventricosum* App. and Wollenw., 1910, and *F. rubiginosum* App. and Wollenw., 1910 [considered a synonym of *F. culmorum* W. G. Sm., 1884, by Wollenweber, 1914 (21)]; *F. subulatum* App. and Wollenw., 1910, as a weak wound parasite under special conditions; *F. orthoceras* App. and Wollenw., 1910, and *F. gibbosum* App. and Wollenw., 1910, as probable causes of tuber-rot.

Jamieson and Wollenweber's description (5) of the powdery dry-rot caused by *F. trichothecioides* is the first description of a definite rot conclusively demonstrated to be caused by a species of *Fusarium* which is sufficiently described in its normal¹ stages to insure certain identification. However, Wilcox, Link, and Pool (17) published a description one year later of the same disease and subnormal stages of the same organism, for which they proposed a new name—i. e., *F. tuberivorum* Wilcox and Link. The examination of material similar to that used by Wilcox and Link from Alliance, Nebr., demonstrated that *F. tuberivorum* is identical with *F. trichothecioides*.

The increasing number of rotting tubers submitted to the Department indicated the existence of several types of a rot not hitherto described which were caused by species of *Fusarium* and focused the author's attention during the past year on a laboratory study of these diseases. The object of this paper is to demonstrate the parasitic nature of certain species of *Fusarium* and to contrast these organisms and the resulting types of deterioration with those already recognized. The economic importance of these rots and the interest manifested by pathologists in a general group of diseases caused by species of *Fusarium* suggested the advisability of a comprehensive treatment of the species known to cause decay as an aid to their diagnoses and ultimate control.

The tuber-rots considered in this investigation are all of the stem-end and wound-parasitic type. They are not sharply differentiated from each other nor from those previously described as caused by the following species: *F. coeruleum*; *F. discolor*, var. *sulphureum*; *F. trichothecioides*. After having made isolations from several hundred submitted specimens of stem-end-diseased tubers and from many more rotting as the result of wound and lenticel invasion or inoculation with known species, the

¹ For a discussion of the idea "normal" as used in this paper, see Wollenweber (21, p. 255-257).

author is convinced that in many cases the only sure way to determine the cause is by cultural studies. In general, specimens of the types of rot developed spontaneously in the field or storage are more characteristic than those produced by inoculation and developed under uniform conditions.

The powdery dry-rot with pink-mycelium-lined cavities caused by *F. trichothecioides* is quite characteristic and not easily confused with the others; the same is true of the rot produced by *F. discolor*, var. *sulphureum*, with its ochreous yellow mycelium, but the rot caused by *F. coeruleum*, in its typical form with external dark-blue mycelium masses and internal blue coloration of the tissues, may be easily confused with some of those herein described unless mature spores are found on the specimen or high cultures are obtained. On some tubers more than one of the wound-parasitic types of *Fusarium* are present; in others, the diagnosis is complicated by the secondary action of bacterial and fungous saprophytes. While the author can in typical cases determine the cause of *Fusarium* rot without the preparation of cultures, the latter is not infrequently the safer method. Our inability to differentiate surely the various rots macroscopically complicates the attempt to differentiate them as types caused by specific organisms.

METHOD OF TESTING PARASITISM

The method employed to demonstrate the wound-parasitic nature of species of *Fusarium* will be outlined in detail before proceeding with the discussion of the several types of tuber-rot and the inoculations with the causal organisms.

Sound tubers as free from skin diseases as possible were selected from the following varieties of potatoes: Burbank, Nette Gem, Early Rose, Idaho Rural, Jersey Peachblow, People's, and Pearl grown at Jerome, Idaho, in 1913 and 1914 and each year kept in cold storage at Washington, D. C., until needed; Irish Cobbler grown in Maine in 1913 and kept in storage through the winter; Green Mountain grown at Arlington, Va., in 1914 and used soon after harvesting.

The selected tubers were washed and disinfected in a solution of 0.5 per cent of formalin, in the majority of the experiments for half an hour, and rinsed in distilled water. Some tubers taken at random were wounded with a large platinum needle, dipped in distilled water, immediately wrapped in waxed paper, and placed in disinfected Altmann incubators. Other tubers were similarly wounded, dipped in distilled-water spore suspensions of the organism to be tested, wrapped, and placed with the controls.

By this method there are chances for secondary invaders, but the used organism is primarily the predominating one. In addition to the control tubers, in every case reisolation, identification in pure culture, and

reinoculation were depended upon to check the work. In many cases transfers of the original strains or of the reisolated ones, or of both, and of any intruders were made to raw, sterile cut potato blocks.

The identification of the closely related species of *Fusarium* employed in this work involved the careful preparation, purification, and morphological study of high cultures. The nutrient media found of most value in obtaining such cultures are as follows: Potato cylinders, rice, stems of cotton (*Gossypium* spp.), and sweet clover (*Melilotus alba*). Agar media were never used, except for plating. As emphasized by Dr. Wollenweber, the vegetable media are very valuable for encouraging characteristic development of species of *Fusarium*.

The control tubers were carefully examined for rot about the wounds. These tubers usually remained as sound as when placed in the incubator, only 4 out of some 140 used as controls having any rot whatever. Sprouting of the inoculated tubers and controls demonstrated their continued viability.

Throughout the incubation periods a maximum humidity was maintained, and necessarily the ventilation was bad. Readings of the temperatures were taken twice daily, and this factor is indicated by the average of all readings obtained from the particular compartment during the stated period. The temperatures were not constant, varying a degree or two above and below the average, but the average as recorded represents very nearly the actual storage temperature, since such fluctuations as occurred were of a temporary nature.

It may be considered by some pathologists that the method is an extreme one: that under the given conditions any organism might be expected to cause a rot. It is believed, however, that the conditions maintained are no more extreme than those to which potato tubers are frequently subjected in field and storage. The following facts tend to establish the validity of the method: (1) Certain organisms—for example, *F. moniliforme* Sheldon, *F. martii* (*sensu strict.*), *Verticillium albo-atrum* Reinke and Berthold, and *Sporotrichum flavissimum* Link—did not cause a rot under these conditions (see p. 201). (2) *F. solani*, *F. vasinfectum*, a species of *Mucor*, and one of *Rhizoctonia* were doubtfully wound-parasitic (see p. 192). (3) The wounded controls remained sound except in a few cases where they were in contact with badly rotted tubers; the same organism was isolated from such controls as from the inoculated tubers in the same compartment. (4) The species of *Fusarium* herein reported as wound parasites grow and rot sterile cut potato blocks in pure culture; none of the intruding organisms (bacteria or fungi) were able to do this, except that in a few cases the submerged part of the block was attacked. These facts, in addition to the experiments, seem to warrant the conclusions reached.

Since the tubers inoculated with the several species of *Fusarium* were treated uniformly and the rots developed by the respective species were

much alike, detailed accounts of the appearances presented are of doubtful value and are eliminated. With every rot-producing species of *Fusarium* included in the experiments the effect was essentially the same—at minimum temperatures, a slow dry-rot; at maximum, a very wet rot, with the tubers completely softened in two or three weeks. Sometimes in the former a mycelium-lined cavity is developed, surrounded by a zone of tissue appearing water-soaked—i. e., a zone of enzymic activity; in other tubers at higher temperatures the same organism proceeds to soften the tuber in a stratiform manner, the several layers reaching across the tuber. Bad-smelling rots did not occur with the species of *Fusarium*. Such rots associated with *Fusarium spp.* were found to be mixed infections. When *Fusarium spp. per se* rot potatoes, an odor suggesting ammonia and trimethylamin is developed.

Rots caused by species of *Fusarium* are commonly spoken of as either “dry-rots” or “wet-rots.” The former are a result of comparatively slow development at low temperatures. The experiments show that any of these organisms capable of causing a rot work more rapidly in an environment of optimum temperature accompanied by high humidity, the tubers developing a wet-rot (see p. 196). Upon drying out, the condition would be termed a “dry-rot.” The two forms grade insensibly into each other, so that neither term is specific. The examination of potato tissues rapidly softening as a result of inoculation with pure cultures of *Fusarium spp.* indicates that the middle lamella is dissolved considerably in advance of the fungus; the hyphæ ramify between the cells, but do not appear to enter them at once. Ultimately the contents of the cells are liberated, and the starch grains become more or less corroded.

It should be noted that the experimental data, revised and grouped under the respective organisms, were obtained through a series of experiments covering a period of more than a year. For example, the data on *F. oxysporum* (see p. 191) were extracted from eight different experiments which included several other species and show at a glance the comparative effect of original and reisolated strains on different varieties of potatoes at sundry temperatures.

In the notes on the artificial inoculations recorded under the respective organisms the history of the various strains is first outlined, followed by a brief consideration of the results in text and tabular form.

CERTAIN FIELD AND STORAGE ROTS OF POTATO TUBERS AND THEIR CAUSE

TUBER-ROT CAUSED BY *FUSARIUM OXYSPORUM* AND *FUSARIUM HYPER- OXYSPORUM*

In a study of a wilt and dry-rot of *Solanum tuberosum*, Smith and Swingle (14) attributed both manifestations to a species of *Fusarium*. After a consideration of the incomplete nature of previous descriptions

of species of *Fusarium* occurring on the potato, they chose the name of the earliest one for their fungus—i. e., *F. oxysporum* Schlechtendahl, 1824. This species was not differentiated from *F. solani* (Mart.) Sacc. and other species occurring on potatoes; although no inoculations are recorded by Smith and Swingle, *F. oxysporum* has been generally accepted as the cause of both the wilt and the dry-rot.

Manns (7) made inoculations with a species of *Fusarium* isolated from the blackened vascular ring and one from dry-rotting tubers, confirming the work of Smith and Swingle (14). He writes as follows (7, p. 316): "In the infection work both of the organisms were wilt producing, bringing about symptoms quite typical with that of the *Fusarium* blight in the field." Tuber-rot as a result of inoculation with a pure culture of his *Fusarium* sp. is not recorded. Like Smith and Swingle (14), he did not consider *F. oxysporum* different from *F. solani*.

Wollenweber (19, 20), after a study of *F. oxysporum* obtained from the vascular system of vines and tubers, was convinced that this species causes the wilt and stem-end ring discoloration, but not a tuber-rot. It simply winters over in the stem end of the tubers. A few quotations show his view regarding this species of *Fusarium*:

* * * the fungus [*F. oxysporum*], a typical xylem inhabitant does not entirely destroy the tuber without the help of tuber rot *Fusaria* or bacteria [20, p. 42].

The fact that *F. oxysporum* causes the wilt of growing potato plants and only uses the xylem of the stem end of tubers for overwintering, without producing a rot of the parenchyma, leads to interesting comparisons * * * [20, p. 42].

Referring to this fungus in his diagnosis, he states that it is a "* * * vascular parasite, cause of wilt disease, but not tuber rot, of *Solanum tuberosum*" (20, p. 28).

To facilitate the arrangement of the species, Wollenweber (19, p. 32) established six provisional sections of the genus *Fusarium* based upon physiological and morphological characters. One of these sections, *Elegans*, comprises the vascular parasites, including *F. oxysporum*.

In general, Wollenweber's views in regard to *F. oxysporum* as indicated above are supported by the writer, but the experience of the last year indicates that these statements should be somewhat modified. The repeated isolation of *F. oxysporum* and related forms of the section *Elegans* from tubers rotting in field and storage, accompanied by the failure in many such cases to obtain any other organisms capable of producing a rot, indicates something more in the nature of this organism than passive hibernation in the vascular ducts of the stem end of potatoes. That the latter may be the chief rôle of the strain of *F. oxysporum* which causes wilt is not doubted. But there are strains of *F. oxysporum* and related forms present in stem-end ring disease and dry-rot which entirely destroy¹ the tubers under the experimental conditions outlined

¹ The fact that *F. oxysporum* is capable of destroying potato tubers is confirmed by Dr. Lon A. Hawkins, of the Bureau of Plant Industry, in unpublished studies on the chemistry of rots of *Fusarium* spp. He employed *F. oxysporum* 3395, a reisolation of strain 2413 (see p. 190).

in another part of this paper. This statement is based upon the results of inoculation work with several strains of *F. oxysporum* isolated from various sources and includes two identified by Wollenweber—i. e., Nos. 1948 and 2413. (See p. 190 and Pl. XV, fig. 3.) The following species and varieties of the section *Elegans* were found to produce tuber-rot in varying degrees: (1) *F. oxysporum*. (2) A related form which differs by producing an abundant pionnotes on potato cylinders. (See p. 206 and Pl. XV, fig. 1, 2.). Morphologically this fungus is identical with *F. hyperoxysporum* (21, p. 268), described as a cause of stem-rot of the sweet potato (*Ipomoea batatas*) by Harter and Field (4, p. 287, 291). The experiments thus far carried out indicate its biological identity—i. e., *F. hyperoxysporum* isolated from *Ipomoea batatas* caused a similar rot under the same conditions. (See p. 192.) (3) *F. vasinfectum* Atkinson, the cause of cotton wilt. (4) Its homologue isolated from wilt of okra (*Abelmoschus esculentus*). The numerous forms of the section *Elegans* type, many of which appear to be morphologically identical but biologically different, require further study, and it is not proposed to enter into a taxonomic consideration of these forms at this time. (See p. 206.)

It seems probable that *F. oxysporum* is incapable of readily penetrating the wall of the xylem. When it enters the vascular ring of the tuber from the wilting mother plant, it hibernates therein during the resting period of the tuber and enters the sprouts with the renewal of vegetative activity. At other times as a wound or lenticel invader, plenty of suitable nourishment is at hand, and it produces a dry-rot or a wet-rot, according to the conditions of temperature and humidity. Possibly as a wound parasite it is without incentive or opportunity to enter the vascular ducts.

Although Smith and Swingle (14) and Manns (7) did not differentiate their *F. oxysporum* form *F. solani* and other species occurring on potato tubers, no evidence has been deduced to show that they were not in the main dealing with the effects of a single species or to prove that *F. oxysporum* does not cause a tuber-rot.

Further notes on *F. oxysporum* as a cause of tuber-rot are given under "Jelly-end rot" and in the experiments.

INOCULATION OF POTATO TUBERS WITH *FUSARIUM OXYSPORUM*, *FUSARIUM HYPEROXYSPORUM*, AND *FUSARIUM VASINFECTUM*

FUSARIUM OXYSPORUM Schlecht.—*F. oxysporum* 2997; isolated on March 10, 1914, from a tuber affected with stem-end ring disease and vascular necrosis, from Everest, Kan. Culture used, 16-day-old pionnotes on stem of *Melilotus alba*. As indicated in Table I, all tubers of the four varieties Jersey Peachblow, Idaho Rural, Early Rose, and People's were rotting after 19 days' incubation at an average temperature of 23.1° C. (See Pl. XV, fig. 3.) The least affected variety

was Idaho Rural. However, many of these were almost completely destroyed, being very mushy and "leaky."¹ The organism was recovered from all varieties, two reisolutions being made from the Rurals.

F. oxysporum 2999; isolated on March 14, 1914, from a tuber with wound-invading brownish dry-rot from Brookings, S. Dak. Culture, 16-day-old pionnotes on stem of *Melilotus alba*. The results were the same as with strain 2997. The organism was recovered in all attempts, reisolutions being made from all varieties except Early Rose.

F. oxysporum 3045; a reisolution of strain 2997 from a rotted tuber of the Idaho Rural variety 20 days after inoculation at 23.1° C. After incubating for 21 days at an average temperature of 25.6° C. all tubers of all varieties—i. e., Netted Gem, Idaho Rural, and People's—showed a deep, progressive rot, a brown zone about the inoculation prick surrounded by a water-soaked area more or less brown in color. The organism was recovered by three isolations.

In a subsequent trial with strain 3045, inoculating the four varieties Idaho Rural, Netted Gem, Burbank, and Pearl with a 1-month-old culture on a stem of *Melilotus alba* and incubating for 37 days at an average temperature of 20.4°, similar results were obtained. Seven reisolutions were identified from this lot.

F. oxysporum 1948; isolated and identified by Dr. Wollenweber from a secondary rot following infection by *Phytophthora infestans*. Material from Honeoye Falls, N. Y., February, 1913. Culture used was 1 month old on stem of *Melilotus alba*. The results at different incubation periods and temperatures are as follows: Ten tubers incubated for 24 days at an average temperature of 24.6° rotted, four slightly decaying in all punctures and six wet-rotting. Organism recovered. One tuber at 18.4° rotted in 51 days, while one at 17.8° failed to decay in this time, but the organism persisted.

F. oxysporum 2413; isolated and identified by Wollenweber in January, 1913, from a potato of the Up-to-Date variety, grown on Potomac Flats, Washington, D. C., in 1912, affected with the ring disease. Cultures used, one on stem of *Melilotus alba* and one on a potato cylinder 25 days old. Result of incubation at 25.7° C. for 14 days: All inoculated tubers decayed, 50 per cent being very badly decomposed with wet-rot; organism recovered by four reisolutions. Two tubers incubated at 17.8° and 18.4°, respectively, for 51 days suffered a rather dry rot; organism recovered.

F. oxysporum 3395; reisolution of strain 2413 from badly rotted Green Mountain potato tuber. Culture used, 4-day-old potato cylinders. Owing to the fact that certain of the tubers were rotting badly, the experiment was concluded before some of the others had started to decay. All of the Pearls, 95 per cent of the Netted Gems, and 50 per cent of the

¹ Orton (9, p. 11) described a soft-rot caused by *Rhizopus nigricans*. Potatoes affected with this disease are called "leaky" or "melters."

Burbanks were rotting after incubation for 25 days at 23.5° C. Four reisolations were made.

In Table I are given the results of inoculations with *F. oxysporum*.

TABLE I.—Results of the inoculation of different varieties of potatoes with original and reisolated strains of *Fusarium oxysporum*

Strain No.	Variety of potato.	Number of tubers.	Incubation period.	Average temperature.	Percentage of tubers rotting.
			Days.	°C.	
2997	Jersey Peachblow..	4	19	23.1	100
	Idaho Rural.....	18	19	23.1	100
	Early Rose.....	5	19	23.1	100
	People's.....	5	19	23.1	100
2999	Jersey Peachblow..	4	19	23.1	100
	Idaho Rural.....	17	19	23.1	100
	Early Rose.....	6	19	23.1	100
	People's.....	6	19	23.1	100
3045>2997	Netted Gem.....	9	21	25.6	100
	Idaho Rural.....	21	21	25.6	100
	People's.....	7	21	25.6	100
	Idaho Rural.....	4	37	20.4	100
3045>2997	Netted Gem.....	4	37	20.4	100
	Burbank.....	4	37	20.4	100
	Pearl.....	4	37	20.4	100
	Green Mountain....	1	51	17.8	0
1948	...do.....	1	51	18.4	100
	...do.....	10	24	24.6	100
	...do.....	1	51	17.8	100
2413	...do.....	1	51	18.4	100
	...do.....	10	14	25.7	100
	Burbank.....	10	25	23.5	50
3395>2413	Netted Gem.....	19	25	23.5	95
	Pearl.....	17	25	23.5	100

>=reisolation of.

FUSARIUM Wollenw.—*F. hyperoxysporum* 3273; isolated in October, 1914, from a soft-rotting Irish potato from Ocean Springs, Miss. (Pl. XV, figs. 1, 2.) Cultures used for inoculation, pionnotes on 56-day-old culture on stem of *Melilotus alba* and a 10-day-old potato cylinder. After 14 days' incubation at an average temperature of 25.7° C. all tubers inoculated with this species were more or less wet-rotted about the inoculation pricks and the lenticels, two tubers being completely softened. The organism was recovered by four reisolations. Fifty-one days' incubation at temperatures ranging from 16.3° to 18.4° gave a slight rot in all. A gradual increase was observed with the increase in temperature. At 18.4° all were rotted, one being completely destroyed. Four reisolations were made.

F. hyperoxysporum 3343; reisolation of strain 3273, from rotting Green Mountain potato tubers 15 days after inoculation at 25.7°. Culture used, a 26-day-old stem of *Melilotus alba* with pionnotes. All of the inoculated tubers of the four varieties Idaho Rural, Netted Gem, Burbank, and Pearl were rotted after an incubation period of 28 and 37

days at average temperatures of 19.7° and 20.4°, respectively. Seven reisolations were identified.

F. hyperoxysporum 3399; isolated from *Ipomoea batatas* from Lincoln, Ark., by Mr. L. L. Harter. Determined by Miss Ethel C. Field and the author. Culture used for inoculation, 20-day-old cotton stem. As given in Table II, after 51 days' incubation at an average temperature of 21.5°, the results were as follows: Of the four inoculated tubers of each of the varieties Idaho Rural, Netted Gem, Burbank, and Pearl 0, 1, 1, and 4 tubers were rotting, respectively. The organism was recovered by four isolations.

F. hyperoxysporum 3489; reisolation of strain 3399. Culture used for inoculation, 8-day-old potato cylinder and rice culture. This strain was considerably more active than the parent strain 3399. All tubers were rotted after an incubation of 25 days at 23.5°. Six reisolations were made.

Table II gives the results of the inoculations with *F. hyperoxysporum*.

TABLE II.—Results of the inoculation of different varieties of potatoes with original and reisolated strains of *Fusarium hyperoxysporum*

Strain No.	Variety of potato.	Number of tubers.	Incubation period.	Average temperature.	Percentage of tubers rotting.
			Days.	°C.	
3273.....	Green Mountain....	4	51	16.3	100
		4	51	17.0	100
		4	51	17.8	100
		4	51	18.4	100
		10	14	25.7	100
3343>3273.....	Idaho Rural.....	4	28	19.7	100
	Netted Gem.....	4	28	19.7	100
	Burbank.....	4	37	20.4	100
	Pearl.....	4	37	20.4	100
	Idaho Rural.....	4	51	21.5	0
3399.....	Netted Gem.....	4	51	21.5	25
	Burbank.....	4	51	21.5	25
	Pearl.....	4	51	21.5	100
	Burbank.....	9	25	23.5	100
3489>3399.....	Netted Gem.....	25	25	23.5	100
	Pearl.....	22	25	23.5	100

>=reisolation of.

FUSARIUM VASINFECTUM Atk.—Inoculations were made with *F. vasinfectum* isolated from cotton and a similar organism from okra to determine whether this species, which is closely related to *F. hyperoxysporum*, would cause a decay of potatoes. Although considerable decomposition occurred in the inoculated tubers, a scrutiny of the data summarized below reveals the nonconclusive nature of the results obtained.

F. vasinfectum 1855; reisolated by Dr. Wollenweber, in December, 1912, from the vascular system of a cotton plant wilting as a result of

inoculation with strain 1733, a reisolation of strain 1635, which in turn was a reisolation of an original strain 1485 obtained from the discolored vascular system of the main root of a wilting cotton plant from Florence, S. C., on June 15, 1912. Culture used, 26-day-old pionnotes on stem of *Melilotus alba*.

F. vasinfectum 3167; reisolation of 1855, on June 19, 1914, from Idaho Rural potato in above experiment, after 25 days' incubation at 25.5° C. Culture used, 19-day-old pionnotes on a potato cylinder.

The results with tubers inoculated with *F. vasinfectum* 1855 after an incubation period of 25 days at an average temperature of 25.5° were as follows: The five tubers of the Netted Gem variety remained sound; one of the three tubers of the Idaho Rural variety and all of the People's variety were rotted, the organism being recovered from both varieties. With strain 3167, one of these reisolations, only 75 per cent of the tubers of the Pearl variety were rotted after 51 days' incubation at an average temperature of 21.5° C. These tubers were attacked only where a comparatively large cut surface had been exposed to the inoculum. The organism was recovered in each attempt, three reisolations being made.

F. vasinfectum 3263; isolated in September, 1914, as a particularly virulent strain of the cotton-wilt fungus from supposedly wilt-resistant cotton obtained in breeding experiments from Denmark, S. C. Culture used, 20-day-old potato cylinder.

F. vasinfectum 3243; isolated on September 5, 1914, from the vascular bundles of a wilting okra plant from Wrightsboro, N. C. Culture used, 20-day-old potato cylinder.

With *F. vasinfectum*, strains 3263 and 3243, the results were less conclusive. In tubers inoculated with the former strain the organism persisted for 41 days at average temperatures of 18.3° and 18.9° without perceptible damage. Of 10 tubers at 23.5° for 41 days, 5 were rotted, the organism being recovered from 3 of them and *F. radicola* being isolated from 2. The organism persisted in the other 5 tubers, though no rot resulted. With strain 3243 the organism persisted for 51 days at 17.8° and 18.4° without damage to the tubers. One tuber at 24.6° for 24 days was badly rotted, and the organism was recovered; of 9 tubers at 23.5° for 41 days, only one rotted. The organism was not recovered, but *F. radicola* was isolated.

In this connection it may be noted that in one experiment (p. 202), which included *F. vasinfectum* 1855 and two strains of *Verticillium albo-atrum* among other organisms, some of the tubers inoculated with the species of *Verticillium* and likewise certain controls rotted; from these the organism used could not be recovered, but *F. vasinfectum* was isolated several times.

Table III gives the data of the inoculations with *F. vasinfectum*.

TABLE III.—Results of the inoculations of different varieties of potatoes with original and reisolated strains of *Fusarium vasinfectum*

Strain No.	Variety of potato.	Number of tubers.	Incubation period.	Average temperature.	Percentage of tubers rotting.
			Days.	°C.	
1855.....	{Netted Gem.....	5	25	25.5	0
	{Idaho Rural.....	3	25	25.5	33
	{People's.....	5	25	25.5	100
3167>1855.....	{Idaho Rural.....	4	51	21.5	0
	{Netted Gem.....	4	51	21.5	0
	{Burbank.....	4	51	21.5	0
	{Pearl.....	4	51	21.5	75
	{Green Mountain.....	1	41	18.3	0
3263.....	{.....do.....	1	41	18.9	0
	{.....do.....	10	41	23.5	50
	{.....do.....	1	51	17.8	0
3243.....	{.....do.....	1	51	18.4	0
	{.....do.....	9	41	23.5	10
	{.....do.....	1	24	24.6	100

>=reisolation of.

JELLY-END ROT AND A TUBER DRY ROT CAUSED BY *FUSARIUM RADICICOLA*

JELLY-END ROT

"Jelly-end" is the very appropriate name applied by growers to potatoes affected with a field rot and a storage rot which annually cause serious losses in the delta lands of California and in the irrigated sections of Oregon and Idaho.

Many of the tubers when dug show the characteristic soft rot at the stem end, the affected portion easily separating from the rest of the tuber (Pl. XVI, XVII). The rot proceeds uniformly until the whole tuber becomes a slimy mass within the entire skin. If allowed to dry out, the skin sometimes persists as a loose attachment at the stem end, or it may shrivel and wrinkle down on the affected part, in this stage suggesting dry rot.

The jelly-end rot is not a new disease, but nothing has been done to establish the cause of the trouble. Orton (9, p. 5), discussing the wilt and dry end-rot of potatoes in California, says: "An early form of this *Fusarium* dry end-rot is frequently met with shortly after digging, and potatoes thus affected are known to buyers as 'jelly-ends.'" Shear (13, p. 6) says: "A serious feature of this disease [wilt] is that it forms a means of entrance for other fungous and bacterial diseases of the tubers, such as 'jelly-end' and dry rot." The examination of specimens from different localities indicates that jelly-end rots may be caused by several species of *Fusarium*. Wollenweber (21, p. 257-258, 264-265) isolated both *F. orthoceras* and *F. radicicola*, and of this disease he says in part (p. 265):

In Watsonville, Cal., in October, 1913, the writer found up to 80 per cent of Burbank potatoes in a large acreage affected by this peculiar soft rot, which is quite different

from that produced by *F. coeruleum* and other species * * *. In tubers with the jelly-end rot *F. orthoceras* is often, but not always, associated with such fungi as *F. radicola*, *Mycosphaerella solani*, *Sporotrichum flavissimum* Lk., *Rhizoctonia*, and also with bacteria.

Concerning *F. radicola*, he says (p. 258):

It is often isolated from Irish potato, especially from dry tubers affected with stem-end dry rot. Sometimes it is associated with other organisms, but frequently seems to invade the tuber from the stolon before a cork layer has been formed * * *. Its presence in the sweet potato suggests that it might require a higher optimum temperature than its related species, such as *F. solani* and *F. martii*.

F. radicola, *F. oxysporum*, *F. moniliforme* Sheldon, and *Rhizoctonia* sp., together with various saprophytic fungi and bacteria, were isolated by the writer from jelly-end rots from Watsonville and Moorland, Cal. *F. orthoceras*, *Mycosphaerella solani*, and *Sporotrichum flavissimum* were not obtained from such tubers.

F. radicola was most frequently obtained from typical "jelly-end" tubers from California and Idaho. Its ubiquitous nature and its behavior in all of the inoculation experiments support the view that it is one of the most important causes of this disease. The relation of this species to other tuber rots is discussed in the paragraph on dry-rot.

The prevalence in California of wilt caused by species of *Fusarium* and the frequency with which *F. oxysporum* was isolated from jelly-end rot suggests the fundamental relationship of this species to the disease. *F. oxysporum* was isolated and identified 24 times from jelly-end rot and stem-end dry-rot tubers from California alone. While often associated with bacteria and fungus saprophytes, in most of these cases it was the only organism secured from the respective tubers which could be regarded as the cause of the condition. It was frequently present in pure culture at the border of rotting and healthy tissues. Whether unaided it produces jelly-end rot under field conditions is not known. A potato tuber from California was diagnosed as ring disease and placed in the incubator. After a period of two months at an average temperature of 18.36° C. a typical jelly-end rot had developed. *F. oxysporum* was the only organism secured from the interior of this tuber at the border of healthy tissue. The inoculation experiments with *F. oxysporum* support the view that it is capable of producing jelly-end rot. *F. radicola* and *F. oxysporum* were also isolated, though not necessarily in association, from rot areas on the side of tubers resulting from wounds and lenticel invasion.

DRY-ROT

F. radicola as a cause of stem-end dry-rot was first obtained in August, 1913, from some tubers submitted from Grassfield, Va. Its widespread occurrence in stem-end dry-rotting tubers may be judged from the following distribution: Hermiston, Oreg.; Watsonville and Sonora, Cal.; Fallon, Nev.; Ocean Springs, Miss.; Jerome, Idaho; Honeove

Falls, N. Y.; Potomac Flats, Washington, D. C.; Arlington, Va.; etc. It enters the stem end of the tubers most commonly, but also invades lenticels and wounds. In some cases the affected tissue is light colored and soft, suggesting bacterial rot—i. e., practically the jelly-end rot. More often in the East it is characterized externally by a firm sunken area with the underlying parenchyma brown to black, dry, tough, and sharply differentiated from the healthy tissue.

This stem-end wound and lenticel dry-rot caused by *F. radicola* may be regarded as a form of jelly-end rot. The organism is one of the causes of jelly-end rot, but the field and storage conditions where it occurs are different. Under conditions of high humidity the rot is of the jelly-end type; where the humidity or temperature is low and the action of the fungus less rapid, dry-rot develops, the affected tissue being more firm and darker colored as a result of drying and oxidation. (See p. 197, Pl. XV, fig. 4, 5.) Both types occur in California, Oregon, and Idaho, sections under irrigation. The dry-rot phase was the one most frequently submitted for diagnosis from other localities—i. e., of presumably slower development at lower temperatures.

INOCULATION OF POTATO TUBERS WITH *FUSARIUM RADICOLA*

F. radicola 2842; isolated in October, 1913, from jelly-end rot of Burbank potato from Middle River, Cal. Unfortunately, the number of tubers in the experiment with this strain was not recorded. About 1 peck of potatoes of the Burbank variety and $\frac{1}{2}$ peck of the Netted Gem variety were used for inoculation and controls. The tubers were incubated at temperatures ranging from 14° to 20.3° ; average lowest compartment, 16.7° ; highest, 18.2° C. After 37 days' incubation only one tuber showed a rot; this was at an average temperature of 18.2° C. The organism was recovered.

The thirty-eighth day after inoculation the remaining tubers were exposed to an average temperature of 22.8° C. for the succeeding 19 days. At this time all inoculated tubers were rotted, all stages of wet-rot and dry-rot being represented. The Netted Gems were more badly affected than the Burbanks. In every case the organism was recovered where the attempt was made, four reisolutions being identified.

F. radicola 2890; isolated in October, 1913, from a jelly-end rotted tuber of the Burbank variety from Watsonville, Cal. (associated with *Rhizoctonia* sp. 2892). Culture used, 9-day-old pionnotes on a stem of *Melilotus alba*. All inoculated tubers showed a progressive rot beginning at the inoculation prick (Pl. XVII) after 20 days' incubation at an average temperature of 23° C. The lenticels were invaded and the sprouts infected and dropping off. Some of the tubers were completely softened, only a slimy mass remaining in the entire skin. The organism was recovered by six reisolutions.

F. radicicola 2890 plus *Rhizoctonia* sp. 2892. The two organisms were used in combination, 14 tubers being inoculated and incubated as above. More advanced decomposition seemed to take place than when *F. radicicola* alone was present. However, the species of *Rhizoctonia* could not be recovered, but *F. radicicola* was reisolated wherever the attempt was made.

F. radicicola 3021; reisolation of 2890 from a Burbank potato 20 days after inoculation with the latter. With this reisolated strain an attempt was made to ascertain the effect of the temperature factor on the action of the organism. The inoculated tubers (Irish Cobbler variety) were badly decomposed at average temperatures of 23.3°, 20.2°, and 19.5° C. At 18.7° the majority were more seriously affected than at lower temperatures; indeed, at 17.5° and 15.1° the effect was a slow dry-rot, while at 12.5° the organism persisted for 88 days without perceptible damage to the host.

F. radicicola 3023. Another reisolation of strain 2890; from lenticel infection after 20 days' incubation at 23° C. All tubers of the three varieties Netted Gem, Idaho Rural, and People's inoculated with this strain and incubated for 21 days at an average temperature of 25.6° were very badly decomposed. The organism was recovered by three isolations.

F. radicicola 2998; isolated March, 1914, from a stem-end ring disease and wound-infected tuber from Fallon, Nev. Culture used, 12-day-old pionnotes on stem of *Melilotus alba*. All tubers inoculated with this strain and incubated 20 days at 23° C. rotted. The organism was recovered.

F. radicicola 3236; isolated in August, 1914, in association with *F. hyerosporum* from a soft-rotting tuber from Ocean Springs, Miss. Culture used, 1-month-old potato cylinder. The results with this strain are as follows: One tuber incubated for 14 days at 25.7° was badly softened with wet-rot. The organism was recovered. Nine tubers at 24.6° for 24 days were slightly rotted in every inoculation prick, one tuber being completely softened with grayish wet-rot. Organism recovered by two reisolations. Sixteen tubers incubated 51 days at temperatures ranging from 16.3 to 18.4° C. gave the following results: At lowest temperature no rot occurred, but the organism had become established; two of the four tubers at 17° were rotting slightly, with the organism established in the others; at 17.8°, two were slightly rotted, with the organism persisting in the others; at 18.4° one tuber was sound and the three others were rotting.

F. radicicola 2862; isolated October, 1913, from jelly-end rot of a tuber of the Burbank variety from Sargent Island near Middle River, Cal. Culture used, 9-day-old pionnotes on stem of *Melilotus alba*. This strain was comparatively inactive, only 12 per cent of the inoculated tubers rotting after 20 days' incubation at 23° C. The organism was recovered.

F. radicola 3319; isolated November, 1913, in association with *Mucor* sp. 3320 from a "leaky" diseased potato tuber from Moorland, Cal. Culture used, 1-month-old pionnotes on a potato cylinder. This strain was similar to 2862, being comparatively inactive. After 51 days' incubation at 21.5 C., only 1 tuber of 16 inoculated developed a rot. No attempt was made to recover the organism.

The results of the inoculations with *F. radicola* are given in Table IV.

TABLE IV.—Results of inoculation of different varieties of potato with original and reisolated strains of *Fusarium radicola*

Species and strain No.	Variety of potato.	Number of tubers.	Incubation period.	Average temperature.	Percentage of tubers rotting.
			Days.	°C.	
<i>Fusarium radicola</i> 2890.	Burbank.....	20	20	23.0	100
<i>Fusarium radicola</i> 2890 and <i>Rhizoctonia</i> sp. 2892.do.....	14	20	23.0	100
<i>Fusarium radicola</i> 3201 > 2890.	Irish Cobbler.....	5	24	23.3	100
		10	88	20.2	100
		10	88	19.6	100
		25	88	18.7	100
		10	88	17.5	100
		6	88	15.1	100
		6	88	12.5	0
<i>Fusarium radicola</i> 3023 > 2890..	{ Netted Gem.....	10	21	25.6	100
	{ Idaho Rural.....	14	21	25.6	100
	{ People's.....	4	21	25.6	100
<i>Fusarium radicola</i> 2998.	Burbank.....	8	20	23.0	100
<i>Fusarium radicola</i> 3236.	Green Mountain....	4	51	16.3	0
		4	51	17	50
		4	51	17.8	50
		4	51	18.4	75
		1	14	25.7	100
<i>Fusarium radicola</i> 2862.	Burbank.....	9	24	24.6	100
		25	20	23.0	12
<i>Fusarium radicola</i> 3319.....	{ Idaho Rural.....	4	51	21.5	0
	{ Netted Gem.....	4	51	21.5	25
	{ Burbank.....	4	51	21.5	0
	{ Pearl.....	4	51	21.5	0

>=reisolation of.

A NEW DRY-ROT CAUSED BY *FUSARIUM EUMARTII*

A type of field and storage rot hitherto undescribed was frequently observed in the examination of potatoes from Pennsylvania during the last two years. The character of this rot is as follows: In mild infection of the stem end the tuber shows externally a darkened sunken area with a greenish luster about the stolon insertion. If a thin slice is cut at this point, the parenchyma and the vascular ring are seen to be browned to varying depths. Some of the bundles are discolored to a greater depth

than the parenchyma and are darker in color, sometimes almost black. In this stage the condition might be mistaken, and probably has been in the past, for stem-end ring disease caused by *F. oxysporum* or *Verticillium albo-atrum*, or for one phase of net necrosis (10, p. 14), which it more closely resembles. By the culture method, however, a species of *Fusarium* is invariably obtained from such tubers at the border of diseased and healthy tissues. The name "*Fusarium eumartii*" is proposed for this fungus.

In the more advanced stages of rot caused by *F. eumartii* the end of the tuber or the entire tuber is involved (Pl. XVIII). According to the humidity and other environmental conditions, the rot is (1) soft and light-brown or (2) dry, corky to friable, and dark-brown to almost black. In general, the rot proceeds uniformly as a sharply differentiated layer easily removable when moist, but close-clinging when dry. In field material the bundles are often discolored as above noted, in advance of the rot. Attempts to isolate the organism from the tips of such bundles usually failed. In the experiments the rot is preceded by a moist water-soaked zone of enzymic activity, from the border of which no organism was obtained. No difficulty was experienced in isolating *F. eumartii* from the border of the discolored tissue and the watery zone.

Considerable care is necessary to differentiate this rot from the one caused by the closely related *F. radicola*. Sometimes the determination is to be decided only by the careful preparation and study of high cultures. The morphological differences between *F. eumartii* and *F. radicola* are discussed on page 205.

F. eumartii is chiefly a stem-end and wound invader, but under favorable conditions the lenticels become infected. The fact that *F. oxysporum* was sometimes obtained in association with this fungus and the further fact that this disease of the tubers is reported on plants described as having symptoms of wilt suggest the probable relationship of *F. oxysporum* to the trouble. A field study of wilt and the relation of *F. oxysporum* to such field rots and storage rots should throw considerable light on the problem.

Attempts to isolate an organism from a type of stem-end necrosis similar to mild cases of invasion with *F. eumartii* often failed. There seems to be a sterile necrosis of the stem end, accompanied by browning of the parenchyma and bundles, which is related to the disease described as net necrosis (10, p. 14, pl. 2). Sometimes this type of stem-end necrosis can be distinguished from slight infection with *F. eumartii* only by the culture method; but when the minute ramifications of the vascular ducts are discolored, resulting in the characteristic phase of net necrosis, it can not be confused with the new type of rot.

This rot was obtained chiefly in Pennsylvania, the following localities representing its known distribution: Tower City and Orwigsburg, Schuylkill County, Pa.; East Greenville, Montgomery County, Euclid,

Butler County, and in Dutchess County, N. Y. To judge from correspondence with growers it is a field rot and a storage rot of considerable importance. Infected tubers placed in storage rot badly the following spring; some of the growers are reported to have lost 50 per cent from dry-rot. Whether unaided *F. eumartii* produces a wilt and a rot as a result of planting infected seed is not known. More likely it is secondary to infection by *F. oxysporum* or *Verticillium albo-atrum* in such cases.

INOCULATION OF POTATO TUBERS WITH FUSARIUM EUMARTII

F. eumartii 2932; isolated on January 3, 1914, from a stem-end dry-rotting tuber (Heath's Medium-Late Surprise variety) from Tower City, Pa. Culture used, 7-day old pionnotes on cotton stem.

F. eumartii 2947; isolated as above on January 15, 1914. Culture used, 7-day-old pionnotes on potato cylinder.

F. eumartii 3040; reisolation of 2947, April 23, 1914, from rotting Idaho Rural potato, 19 days after inoculation at 23.1° C. Cultures used, 22-day-old pionnotes on potato cylinder, and in a subsequent trial 2-months-old cultures on rice, *Melilotus alba*, and cotton stems.

F. eumartii 2958; isolated on January 28, 1914, as recorded in Nos. 2932 and 2947. Culture used, 7-day-old pionnotes on potato cylinder.

All tubers of the five varieties mentioned which were inoculated with the several original and reisolated strains of this species of *Fusarium* showed a progressive rot beginning at the points of inoculation in each case; many of the lenticels were invaded, sunken, and with the subjacent parenchyma browned. People's variety was the most susceptible, the others being affected in the order named—Early Rose, Jersey Peachblow, Netted Gem, and Idaho Rural (Pl. XIX). However, even in the last-mentioned variety there was 100 per cent of infection about the inoculation pricks and lenticel invasion of all tubers. Some of the inoculated tubers were completely softened; others showed a dark-brown zone about the inoculation prick, surrounded by an extensive watery zone of softened tissue. At low temperatures a typical slow dry-rot was produced. The respective organisms were recovered in every attempt made: Nos. 2932 and 2947 from all varieties used; 2958 from the Idaho Rurals; 3040 in first trial, one reisolation from the Idaho Rurals, and one from the Netted Gems; in a later experiment five reisolations were made from the Idaho Rural variety.

Table V gives the results of the inoculations with *F. eumartii*.

TABLE V.—Results of the inoculation of different varieties of potatoes with original and reisolated strains of *Fusarium eumartii*

Strain No.	Variety of potato.	Number of tubers.	Incubation period.	Average temperature.	Percentage of tubers rotting.
			Days.	°C.	
2932.....	Jersey Peachblow...	3	19	23.1	100
	Idaho Rural.....	14	19	23.1	100
	Early Rose.....	4	19	23.1	100
	People's.....	4	19	23.1	100
2947.....	Jersey Peachblow...	4	19	23.1	100
	Idaho Rural.....	19	19	23.1	100
	Early Rose.....	4	19	23.1	100
	People's.....	4	19	23.1	100
3040>2947.....	Netted Gem.....	9	21	25.6	100
	Idaho Rural.....	14	21	25.6	100
	People's.....	5	21	25.6	100
		15	65	13.8	100
3040.....	Idaho Rural.....	15	65	17.2	100
		15	65	18.6	100
	Jersey Peachblow...	3	19	23.1	100
2958.....	Idaho Rural.....	18	19	23.1	100
	Early Rose.....	3	19	23.1	100
	People's.....	3	19	23.1	100

>=reisolation of.

CONTROL INOCULATIONS OF POTATO TUBERS

In order to ascertain whether any organism at random would cause a decay of potato tubers under the conditions used to establish the wound-parasitic property of the species mentioned, certain species of *Fusarium* and other organisms inhabiting potato tubers were included in the experiments. The following organisms were used for this purpose: *F. martii*, *F. solani*, *F. moniliforme*, *Verticillium albo-atrum*, *Sporotrichum flavissimum*, a species of *Mucor*, and a species of *Rhizoctonia*. The notes on the effect of these organisms on different varieties of potatoes at sundry temperatures are extracted from the several experiments and grouped according to organism as a support of the method. It may be mentioned in this connection that certain strains of *F. radicola* (Nos. 2862 and 3319) were found to be comparatively inactive under conditions identical with those in which other strains were most virulent.

INOCULATION OF POTATO TUBERS WITH CERTAIN SPECIES OF FUSARIUM AND OTHER TUBER-INHABITING ORGANISMS

F. solani 176; isolated in 1908 by Dr. Wollenweber at Dahlem, near Berlin, Germany, from a potato tuber. Used for the original description of this species by Appel and Wollenweber (1, p. 77). Culture used, 2-months-old pionnotes on potato cylinder. After 51 days at an average temperature of 21.5° C., this organism had attacked only 50 per cent of but one variety, Pearl, and then only where a large cut surface was

exposed. In other words, but 2 tubers of 16 inoculated were rotted. From the two affected tubers *F. solani* was recovered once, *F. radiculicola* was isolated twice, and *F. oxysporum* once.

F. martii 186; isolated from *Pisum sativum* in April, 1910. Sent to Dahlem, Germany, by Miss J. Westerdijk, of Amsterdam, Netherlands, as *F. vasinfectum*, var. *pisi* Van Hall; determined by Dr. Wollenweber. Culture used, 2-months-old pionnotes on potato cylinder. None of the 16 tubers inoculated was affected after 51 days' incubation at an average temperature of 21.5° C.

F. moniliforme 3321; isolated on November 3, 1914, in association with *F. radiculicola* 3319 and *Mucor* sp. 3320 from a "leaky" (see footnote, p. 190) tuber from Moorland, Cal. Culture used, 1½-months-old cotton stem culture. Of the 16 tubers inoculated, none was rotted after 41 days at an average temperature of 21.5° C.

Verticillium albo-atrum 1717 and 2784. The former strain was isolated by Dr. Wollenweber in September, 1912, from the discolored vascular bundles of wilting okra plant from Monetta, S. C. Strain 2784 was isolated on August 28, 1913, from a wilting potato plant of the Rural variety from Greeley, Colo. After an incubation period of 25 days at an average temperature of 25.5° C. the tubers of the Netted Gem and Idaho Rural varieties inoculated with the respective strains remained sound. The tubers of the People's variety inoculated with these strains were badly rotted in both cases. The organisms could not be recovered, but *F. vasinfectum* was isolated. Tubers inoculated with the latter species were in the same compartment.

Sporotrichum flavissimum 1455; isolated and determined by Dr. Wollenweber in May, 1912; from a hollow Irish Cobbler potato from Arlington, Va. Culture used for inoculation, 2-weeks-old potato cylinder. Of 12 tubers inoculated with the organism and incubated for 20 days at 23° C., none was rotted.

Mucor sp. 3320; isolated on November 3, 1914; from same source and in association with *F. moniliforme* 3321 and *F. radiculicola* 3319. Culture used, 2-months-old fruiting culture on cotton stem. Two tubers out of 16 inoculated with this organism were rotted after incubating for 51 days at 21.5° C. From these the organism was recovered by one reiso-lation, and *F. oxysporum* and *F. vasinfectum* were isolated each once. Tubers inoculated with the latter species were in the same compartment.

Rhizoctonia sp.; for inoculation results with this organism see p. 197.

In Table VI are given the results of the inoculations with the species of *Fusarium* and other potato-inhabiting organisms.

TABLE VI.—Results of the inoculation of different varieties of potato tubers with certain species of *Fusarium* and other tuber-inhabiting organisms

Species and strain No.	Variety of potato.	Number of tubers.	Incubation period.	Average temperature.	Percentage of tubers rotting.
			Days.	°C.	
<i>Fusarium solani</i> 176.	Idaho Rural.....	4	51	21.5	0
	Netted Gem.....	4	51	21.5	0
	Burbank.....	4	51	21.5	0
	Pearl.....	4	51	21.5	^a 50
<i>Fusarium martii</i> 186.	Idaho Rural.....	4	51	21.5	0
	Netted Gem.....	4	51	21.5	0
	Burbank.....	4	51	21.5	0
	Pearl.....	4	51	21.5	0
<i>Fusarium moniliforme</i> 3321.	Idaho Rural.....	4	41	21.5	0
	Netted Gem.....	4	41	21.5	0
	Burbank.....	4	41	21.5	0
	Pearl.....	4	41	21.5	0
<i>Verticillium albo-atrum</i> 1717.	Netted Gem.....	4	25	25.5	0
	Idaho Rural.....	4	25	25.5	0
	People's.....	5	25	25.5	^a 100
	Netted Gem.....	4	25	25.5	0
<i>Verticillium albo-atrum</i> 2784.	Idaho Rural.....	3	25	25.5	0
	People's.....	5	25	25.5	^a 60
	Burbank.....	12	20	23.0	0
<i>Sporotrichum flavissimum</i> 1455.	Idaho Rural.....	4	51	21.5	0
	Netted Gem.....	4	51	21.5	^a 25
	Burbank.....	4	51	21.5	0
	Pearl.....	4	51	21.5	^a 25

^a The respective organism is doubtfully the cause, as in each case wound-parasitic species of *Fusarium* were isolated in association. See text.

TAXONOMIC ARRANGEMENT AND DIAGNOSTIC CHARACTERS OF IMPORTANT ROT-PRODUCING SPECIES OF *FUSARIUM*

FUSARIUM Link

The sections Martiella, Elegans, and Discolor provisionally established by Wollenweber (19, p. 32; 20, p. 28) include the species of *Fusarium* causing tuber-rot known to be economically important. Certain other species—namely, *F. ventricosum*, *F. gibbosum*, *F. culmorum*, *F. orthoceras*, and *F. subulatum*—reported by Wollenweber (19, 20) as weak wound parasites of the Irish potato are not included in the following arrangement of species. *F. solani*, the type species of the section Martiella, is listed because of its ubiquitous occurrence on potatoes as well as on roots and tubers of other plants. Subnormal conidia of *F. coeruleum*, *F. radiculicola*, and *F. eumartii* are easily confused with those of *F. solani*. The form, size, and septation of normal conidia must be depended upon for differentiation.

A. SECTION MARTIELLA

[Species in this section are *F. solani* (Mart.) Sacc., *F. martii* App. and Wollenw., *F. eumartii*, n. sp., *F. coeruleum* (Lib.) Sacc., and *F. radicola* Wollenw.]

1. *Fusarium solani* (Mart.) Sacc. (1, p. 77).

Conidia normally triseptate (Pl. XIV, fig. 3) up to 100 per cent, occurring in pionnotes and sporodochia,¹ averaging 30 to 40 by 5 to 6 μ . Limits of normal triseptate conidia: 25 to 45 by 4.5 to 6.5 μ . Seldom 2 and 4, exceptionally 1 and 5 septate (limits: 1-septate, 15 by 4 μ minimum; 5-septate, 59 by 6.5 μ maximum; greatest width, 7 μ ; highest septation, 7.) Conidial mass brownish white, becoming brown in age; often greenish as a result of infiltration with greenish blue pigment from the plectenchymatic mycelium. Chlamydospores terminal, intercalary, and conidial; unicellular, round or pear-shaped, 8.5 by 8 μ ; 2-celled with constriction at cross wall, 12 by 7.75 μ ; smooth, rarely in chains or clumps.

Habitat.—On decaying tubers and roots of plants and in the soil. Isolated from species of *Solanum*, *Citrullus*, *Cucumis*, *Cucurbita*, *Lycopersicon*, *Pinus*, *Hibiscus*, *Avena*, *Zea*, *Triticum*, *Panax*, *Citrus*, *Pelargonium*. Collected by various investigators and identified by Wollenweber and Carpenter.

F. solani (*sensu strict.*) is regarded as a saprophyte, but apparently it acts as a weak wound parasite under exceptionally favorable conditions.

2. *Fusarium coeruleum* (Lib.) Sacc. (1, p. 90).

Conidia normally triseptate (Pl. XIV, fig. 5), averaging 30 to 40 by 4.5 to 5.5 μ (limits of normal triseptate conidia: 23 to 47 by 4.25 to 6 μ); seldom 4 and 5 septate (limits: triseptate, 23 by 4.25 μ minimum; 7-septate, 58 by 5.75 μ maximum). Conidial mass brownish white and yellow ochre to reddish ochre. Plectenchymatic stroma chiefly violet to indigo blue and bluish black; by infiltration with the latter color the conidial masses may become bluish green, as in other species of the section Martiella. *F. coeruleum* is the only species of the section having reddish ochre conidial masses. Chlamydospores as in other species of the section.

Habitat.—On tubers of *Solanum tuberosum*. Established as a cause of tuber rot in this country and in Europe by Wollenweber (20, p. 44). Determined by Dr. Wollenweber and the writer in material from the following localities: Ottawa, Canada; Houlton, Me.; Rhinebeck, N. Y.; Fredericksburg, Md.; Norfolk, Va.; Parkersburg, W. Va.; Donnybrook, N. Dak.; Idaho Falls, Idaho; Potlatch, Wash.; and several places in Oregon.

3. *Fusarium eumartii*, n. sp.

F. eumartii isolated from the Pennsylvania dry-rot agrees with Appel and Wollenweber's (1, p. 78-84) diagnosis of *F. martii* except in certain details of the conidia. The latter in the new species are higher septate and have a somewhat larger average size (Pl. XIV, fig. 4). Normally 4 to 6 septate, averaging 54 to 75 by 5.5 to 6.6 μ (limits: 50 to 80 by 5 to 7.2 μ). Largest conidia 85 by 7.2 μ (7 and 8 septate). Percentages of variously septate conidia, average sizes and limits as found in a 10-day-old pionnotes on *Melilotus alba* and in a 15-day-old pionnotes on cotton are given in Table VII.

¹ For definition of these terms see Wollenweber, H. W. (20, p. 24).

TABLE VII.—Percentages of variously septate normal conidia, average sizes, and limits of size as found in a 10-day-old pionnotes on *Melilotus alba* and in a 15-day-old cotton pionnotes of *Fusarium eumartii*.

10-DAY-OLD PIONNOTES ON MELILOTUS ALBA

Septation.	Percentage of conidia.	Average size of conidia.	Limits.
		μ	μ
3.....	7
4.....	20	54.4 by 5.6....	51 to 54.4 by 5.1 to 6.1.
5.....	50	63.7 by 5.8....	59.5 to 69.7 by 5.4 to 6.1.
6.....	8	69.7 by 6.3....	66.3 to 71.4 by 6.1 to 6.8.
7.....	15	71.6 by 6.5....	68 to 76.5 by 5.9 to 6.8.

15-DAY-OLD PIONNOTES ON COTTON

3.....	5
4.....	17
5.....	58	62.9 by 6.1....	56 to 76.5 by 5 to 6.8.
6.....	18	73.2 by 6.6....	51 to 81.6 by 5.9 to 7.2.
7.....	2	79.9 by 6.6....	74.8 to 85 by 6.3 to 6.8.
8.....	Rare.	85 by 6.8.....

The formation of pigment in *F. eumartii* (Pl. A, fig. 6-8) and *F. radicola* is much the same as that in *F. solani*, only more gorgeous. The conidial color fluctuates between brownish white and bright brown; by infiltration of the greenish blue plectenchymatic pigment the conidial mass becomes gray, blue-green, to brown and a dark mixed color. The plectenchymatic stroma is weakly developed or lacking, and therefore the pionnotes lies naked on the substratum. The chlamydospores, 7 to 10 μ in diameter, agree with those in other species of this section.

F. eumartii causes a rot of potatoes in experiments, while *F. martii* is said to be a saprophyte (20, p. 30). This statement was confirmed with *F. martii* 186 collected in Germany. The new species agrees more closely with *Fusisporium solani* Martius (8) in the size of conidia than does *F. martii*.

F. radicola and *F. eumartii* are very closely related to *F. martii* with respect to average size and septation of normal conidia and occupy the same relative positions on either side of the last-mentioned species as a type. In average measurements the conidia of *F. radicola* are approximately 30 per cent shorter and 20 per cent narrower than those of *F. martii* (*sensu strict.*), while *F. eumartii* is larger in about the same proportion. *F. radicola* is typically triseptate, *F. martii* 3- to 4-septate, and the new species 5- to 6-septate. Similar constant varieties of certain other species are known—e. g., of *Fusarium solani*.

Habitat.—On decaying tubers of *Solanum tuberosum* from Pennsylvania and New York. Cause of potato dry-rot and wet-rot.

4. *Fusarium radicola* Wollenw. (21, p. 257-258).

The conidia of this species are normally triseptate, averaging 30 to 45 by 3.75 to 5 μ ; narrower than in *F. solani*, *sensu strict.* (Pl. XIV, fig. 3), and shorter and fewer septate than in *F. martii* and *F. eumartii* (Pl. XIV, fig. 4). The plectenchymatic mycelium, as in the two latter species, is olive colored on potato cylinders, shading to green and brown. Pionnotes on potato cylinders, cotton, and stems of *Melilotus alba* brownish white to blue and verdigris (Pl. A, fig. 6-8). Pigment formation the same as in *F. martii* and *F. eumartii*. Chlamydospores as in other species of the section.

Habitat.—On partly decayed tubers and roots of plants. Cause of potato dry-rot and jelly-end rot. Identified from the following: *Ipomoea batatas* (collected by Mr. L. L. Harter); *Musa sapientum* (collected by Mr. S. F. Ashby, Jamaica, Porto Rico); *Cucumis sativus* (collected by Mr. F. V. Rand, West Haven, Conn.); soil (collected by Mr. F. C. Werkenthin, Austin, Tex.).

B. SECTION ELEGANS

[Species in this section are *F. oxysporum* Schlecht., *F. hyperoxysporum* Wollenw., *F. vasinfectum* Atk., *F. tracheiphilum* Sm., *F. niveum* Sm., *F. lycopersici* Sacc., *F. conglutinans* Wollenw., *F. redolens* Wollenw., *F. orthoceras* App. and Wollenw., *F. orthoceras*, var. *triseptatum* Wollenw., *F. batatatis* Wollenw.]

1. *Fusarium oxysporum* Schlecht. (20, p. 28).

2. *Fusarium hyperoxysporum* Wollenw. (21, p. 268).

F. oxysporum (Pl. XIV, fig. 1) is not sharply differentiated morphologically from several species of this section—namely, *F. hyperoxysporum*, *F. vasinfectum*, *F. tracheiphilum*, *F. lycopersici*, and *F. niveum*. *F. hyperoxysporum* forms a perfect pionnotes in contrast to the reduced pionnotes in *F. oxysporum* (Pl. A, fig. 1-5). According to Harter and Field (4, p. 296), it is different biologically in that it causes a stem-rot of *Ipomoea batatas* and is not infectious on young plants of *Solanum tuberosum*, while *F. oxysporum* causes a wilt of the latter host but does not attack the former (21, p. 268). Both develop a lilac odor on starchy media. However, this character is of doubtful specific value since non-odor-forming strains of *F. oxysporum*, *F. hyperoxysporum*, and *F. vasinfectum* have been isolated, and some of the odor-forming strains temporarily lose this property in culture.

F. tracheiphilum, the cause of a wilt of species of *Vigna*, is without pionnotes and odor. *F. vasinfectum*, the cause of a wilt of cotton, develops a perfect pionnotes of an ocherous-salmon color; on potato cylinders in subdued light this color becomes slightly purple. Typically a strong lilac odor is present on starchy media. A non-odor-forming strain was designated *F. vasinfectum*, var. *inodoratum*, by Wollenweber (20, p. 29). *F. lycopersici*, the cause of a wilt of *Solanum lycopersicum*, differs from *F. oxysporum* in having conidia of a little larger average size and produces colorless sclerotial plectenchymatic masses in contrast to the bluish masses of this sort in *F. oxysporum*, etc. No odor is developed. *F. niveum*, to which the wilt of species of *Citrullus* is attributed, differs from *F. lycopersici* in forming blue sclerotial bodies on potato cylinders; from *F. oxysporum* in having larger conidia and no odor.

It is possible to determine the six above-mentioned species by morphological characters alone. Although a knowledge of the host of the particular species to be determined is not necessary, such information greatly facilitates the work. In spite of the fact that each of these forms seems to cause a wilt on one particular host, it should be pointed out that too much dependence on the value of the host in descriptions of species has led to the present confusion in the nomenclature of the form genus *Fusarium*.

A species of *Fusarium* causing a field soft-rot of Irish potatoes in Mississippi (Pl. XV, fig. 1, 2) was morphologically identical with *F. oxysporum* (Pl. XIV, fig. 1), but developed a perfect pionnotes on potato cylinders (Pl. A, fig. 4); thus, it must be identical with *F. hyperoxysporum*, the cause of stem-rot of the sweet potato. Inoculation with *F. hyperoxysporum* isolated by Harter and Field from the latter host resulted in complete destruction of the tubers (see No. 3399 and reisolation of same, No. 3489, p. 192), indicating the truth of the hypothesis.

Further cross-inoculation work carefully controlled by morphological studies should demonstrate whether all of the above-mentioned species of this section are biologically distinct; whether, for example, *F. hyperoxysporum* differs sufficiently from *F. oxysporum*, on the one hand, and *F. vasinfectum*, on the other, to be entitled to the rank of species.

C. SECTION DISCOLOR

[Species in this section are *F. discolor* App. and Wollenw.; *F. discolor*, var. *sulphureum* (Schlecht.) App. and Wollenw.; *F. culmorum* (W. G. Sm.) Sacc. (syn., *F. rubiginosum* App. and Wollenw.); *F. trichothecioides* Wollenw.; and *F. incarnatum* (Rob.) Sacc.]

1. *Fusarium discolor*, var. *sulphureum* (Schlecht.) App. and Wollenw. (1, p. 115-118).

F. discolor, var. *sulphureum*, is morphologically the same as *F. discolor* App. and Wollenw. (1, p. 114). Normal conidia (Pl. XIV, fig. 6) 3- to 5-septate, 23 to 39 by 4.5 to 5.5 μ (limits: 16 to 48 by 3.5 to 6 μ); exceptionally 1- and 2-septate. True chlamydospores are rare. Conidial masses ochreous to ochreous-orange. Differs from *F. discolor* in the color of the plectenchymatic mycelium, which never becomes carmine-red (Pl. B.), but changes from ochreous to yellow (egg-yellow to sulphur-yellow, which color permeates the aerial mycelium and conidial masses).

Habitat.—In hollows of potato tubers. Established by Dr. Wollenweber as the cause of a tuber-rot in Germany. It was isolated from decaying tubers from Newell, S. Dak., and identified by Dr. Wollenweber. The writer also identified it in similar material from Cresbard, S. Dak., and in tubers from the North Dakota Agricultural College (collected by Mr. D. G. Milbrath).

2. *Fusarium trichothecioides* Wollenw. (5, p. 146-152).

F. trichothecioides, in contrast to the other species of the section *Discolor*, forms two sorts of conidia: (1) The comma type, formed as a slightly curved comma ellipsoidally rounded on both sides; and (2) the normal macroconidia, typical of the section. The plectenchymatic mycelium and conidial masses are rosy white, in contrast to the carmine¹ mycelium in *F. discolor* (Pl. B, fig. 1-3) and the ochreous-yellow mycelium in *F. discolor*, var. *sulphureum* (Pl. B, fig. 4-6). The conidial masses in both the last-named species are ochreous orange.

Habitat.—Dry-rotting tubers of *Solanum tuberosum*, causing decay, especially under storage conditions. Geographic distribution: Spokane, Wash.; St. Paul, Minn.; Dayton, Iowa; Alliance, Nebr.; Spearfish, S. Dak. (Jamieson and Wollenweber). The following localities are added to the above: Jerome and Idaho Falls, Idaho; Newell, S. Dak.; and Sioux City, Iowa.

SUMMARY

(1) A new stem-end and wound-invading dry-rot of the Irish potato annually causing serious damage in Pennsylvania is caused by a species of *Fusarium* for which the name "*Fusarium eumartii*" is proposed.

(2) Another widely prevalent dry-rot similar to the above is caused by *F. radicicola* Wollenw.

(3) *F. radicicola* and *F. oxysporum* are most commonly associated with the so-called "jelly-end" rot, annually a serious trouble in the tule lands of California. The former seems to be the cause in most cases, but the fundamental relationship of *F. oxysporum* to this and other tuber-rots should not be overlooked.

(4) Experimental inoculations show that *F. oxysporum* and *F. hyperoxysporum*, species of the section *Elegans*, which has been reported as containing purely vascular parasites, are capable of entirely destroying potato tubers.

(5) *F. oxysporum* is the cause of certain types of tuber-rot.

¹ Jamieson and Wollenweber (5) give "purple" mycelium through error.

(6) *F. radiculicola* caused no rot at 12° C. A constant storage temperature below 50° F. would prevent the action of *F. radiculicola*, *F. eumartii*, and *F. oxysporum*.

(7) The following species of *Fusarium* are added to those known to cause tuber-rot through wound infection: *F. radiculicola* Wollenw.; *F. eumartii*, n. sp.; *F. oxysporum* Schlecht.; and *F. hyperoxysporum* Wollenw.

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PLATE A

Fusarium spp. on vegetable media:

Fig. 1-3 and 5.—*Fusarium oxysporum* Schlecht. 3045. 1, Twenty-one-day-old culture on potato cylinder showing typical bluish green sclerotial masses, no pionnotes. 2, Eighteen-day-old culture on stem of *Melilotus alba* with pionnotes. 3, Eighteen-day-old rice culture with typical coloration of the section Elegans. 5, Thirty-day-old cotton-stem culture with sporodochia.

Fig. 4.—*F. hyperoxysporum* Wollenw. 3343. Thirty-one-day-old culture on potato cylinder with development of pionnotes. Cultures on the three other media are as illustrated for *F. oxysporum* (fig. 1-3, 5).

Fig. 6-8.—*F. radicola* Wollenw.; illustrates equally well *F. martii* and *F. eumartii*. 6, Potato cylinder 34 days old with pionnotes brown to verdigris. 7, Seventeen-day-old culture on stem of *Melilotus alba* with pionnotes and immature sporodochia. 8, Rice 28 days old, with pionnotes on upper surface. Coloration of the section Martiella.





Shull

PLATE B

Fusarium spp. on vegetable media:

Fig. 1-3.—*Fusarium discolor* Appel and Wollenw. 153, showing typical color reactions of this type species of the section *Discolor*. This section includes *F. trichothecioides* and *F. discolor*, var. *sulphureum*, both of which differ from the type in color reactions. 1, Potato cylinder 11 days old, showing carmine red pigmentation of the plectenchymatic mycelium. 2, Culture on cotton stem 35 days old, showing sporodochia and pionnotes drying out. 3, Rice culture 11 days old.

Fig. 4-6.—*F. discolor*, var. *sulphureum* (Schlecht.) Appel and Wollenw. 154. 4, Ocherous-orange pionnotes on 11-day-old potato cylinder. 5, Sporodochia on 39-day-old cotton-stem culture. 6, Rice culture 11 days old.

PLATE XIV

Fig. 1.—*Fusarium oxysporum* Schlecht: A, Normal conidia. B. Swollen conidia, the first one exceptionally long and high septate. C, Conidio-chlamydospores. D. Young intercalary and terminal chlamydospores. $\times 1,000$.

Fig. 2.—*F. radicicola* Wollenw. Normal conidia. $\times 1,000$.

Fig. 3.—*F. solani* (Mart.) Sacc. Type species of the section Martiella. Normal conidia. $\times 1,000$.

Fig. 4.—*F. eumartii*, n. sp. Normal conidia. $\times 1,000$.

Fig. 5.—*F. coeruleum* (Lib.) Sacc. Normal conidia. $\times 1,000$.

Fig. 6.—*F. discolor*, var. *sulphureum* (Schlecht.) App. and Wollenw. Normal conidia. $\times 1,000$.

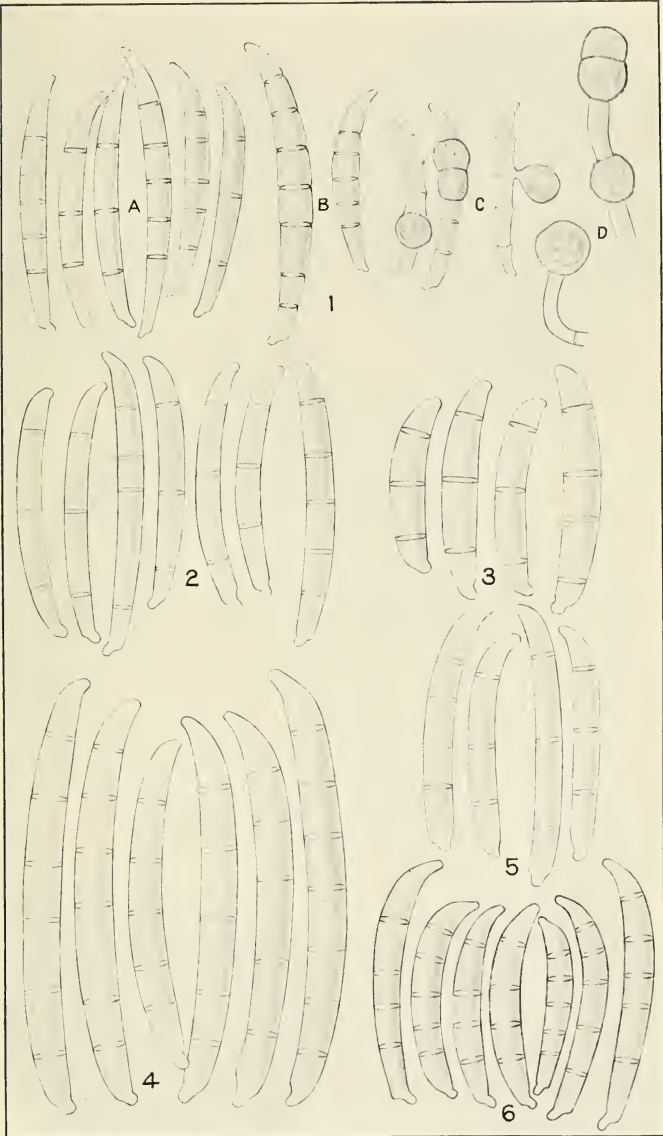


PLATE XVI

Two "jelly end" tubers from Moorland, Cal., showing external views and longitudinal sections.



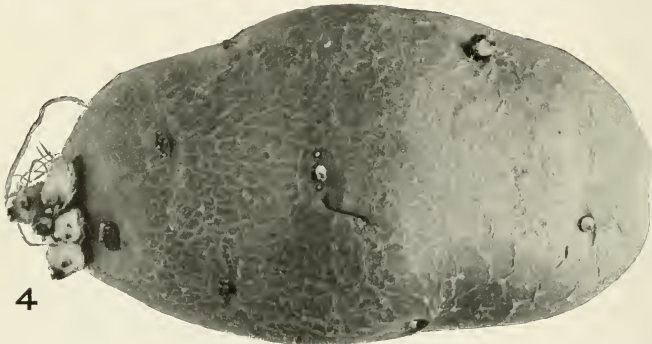
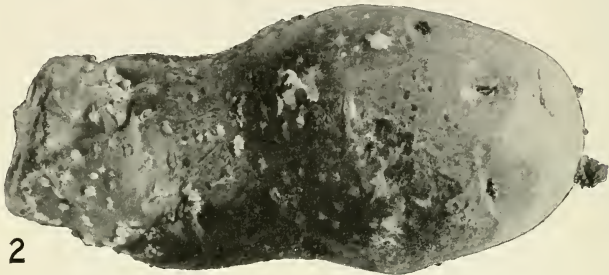


PLATE XVII

"Jelly-end" rot produced by inoculation with *Fusarium radicola* Wollenw.:

Fig. 1.—Control potato tuber.

Fig. 2, 3, 4.—Potato tuber inoculated with *F. radicola* 2890; isolated from material similar to that shown in Plate XVI.

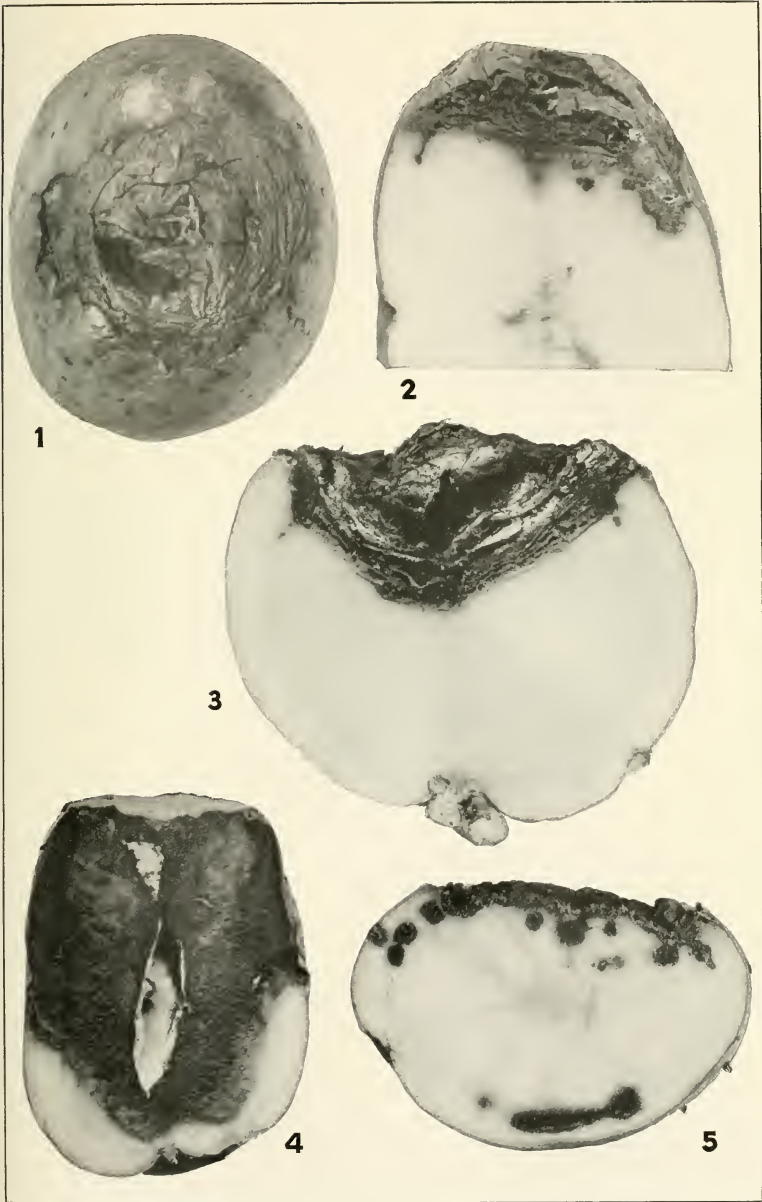
PLATE XVIII

Tuber-rot from Pennsylvania caused by *Fusarium eumartii*, n. sp.:

Fig. 1, 2.—External and sectional view of the same potato tuber. The spots in the center of figure 2 are not pertinent.

Fig. 3, 4.—Sectional views of other potato tubers.

Fig. 5.—A cross section of a potato tuber showing how the fungus frequently follows the tissue adjacent to the bundle ring.



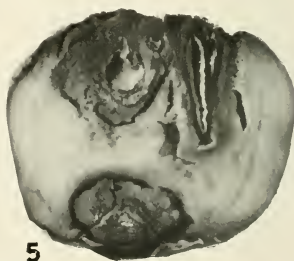
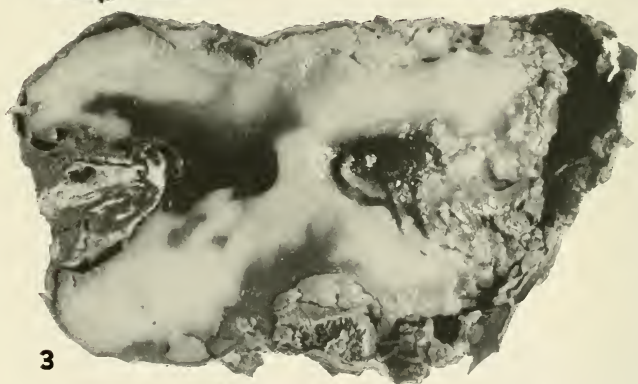


PLATE XIX

Tuber-rot produced in the laboratory with *Fusarium eumartii*, n. sp., and control potato tuber:

Fig. 1, 2.—Control.

Fig. 3.—Potato tubers showing a soft-rot, as a result of rapid development. Incubation period 19 days at room temperature. People's variety.

Fig. 4, 5.—Potato tubers selected to illustrate the type of rot in slower development. Jersey Peachblow variety.

INFECTION EXPERIMENTS WITH TIMOTHY RUST

By E. C. STAKMAN, *Head of the Section of Plant Pathology and Bacteriology*, and
LOUISE JENSEN, *Mycologist, Division of Botany and Plant Pathology, Department
of Agriculture, University of Minnesota*

INTRODUCTION

There is some diversity of opinion as to whether or not timothy rust should be regarded as a distinct species. Eriksson and Henning (2, p. 140-142)¹ in 1894 designated it "*Puccinia phleipratensis* Eriks. u. Henn." Johnson (4) decided that timothy rust in this country was the same as that in Sweden and favors giving the fungus specific rank. Kern (5, 6), on the other hand, thinks it should be considered as a physiological species, or, at most, a variety or subspecies.

It is therefore of interest to know the infection capabilities of the rust. Eriksson and Henning (3, p. 136-141), reported the successful infection of rye (*Secale cereale*) and oats (*Avena sativa*), but none of wheat (*Triticum vulgare*) or barley (*Hordeum vulgare*). Johnson (4, p. 9) obtained results confirming those of Eriksson and Henning. Johnson also succeeded in successfully infecting a number of grasses. He found that the rust would not transfer directly to barley, but if transferred first to oats and then to barley infection resulted. In the same way *Dactylis glomerata* acted as a bridging form between timothy and wheat. Mercer (7) was unable to obtain successful infection on wheat, rye, and various grasses as a result of inoculations made with timothy-rust urediniospores.

The inoculations made by the writers were all on seedlings. The leaves were first thoroughly moistened either with an atomizer or by rubbing water on with the fingers. The spores were applied with a flat inoculating needle. The plants were then placed in shallow pans of water and kept covered with bell jars for 48 hours. The grass seeds were obtained from the Minnesota Seed Laboratory. The following varieties of cereals were used: Oats, Improved Ligowa, Minn. No. 281; barley, Manchuria, Minn. No. 105; wheat, Bluestem, Minn. No. 169; rye, Swedish, Minn. No. 2.

RESULTS OF INOCULATIONS

The writers made a number of inoculations with timothy-rust urediniospores, the results of which are given in Table I.

¹ Reference is made by number to "Literature cited," p. 216.

TABLE I.—Results of inoculations with timothy-rust urediniospores on cereals and grasses

Date of inoculation.	Source of urediniospores.	Plant inoculated.	Number of leaves inoculated.	Number of leaves infected.
Dec. 18, 1914.....	<i>Phleum pratense</i>	<i>Triticum vulgare</i>	37	0
Dec. 24, 1914.....	do.....	do.....	20	0
Dec. 29, 1914.....	do.....	do.....	41	0
Jan. 26, 1915.....	do.....	do.....	52	0
Dec. 18, 1914.....	do.....	<i>Avena sativa</i>	25	3
Dec. 24, 1914.....	do.....	do.....	20	0
Jan. 9, 1915.....	do.....	do.....	23	14
Jan. 17, 1915.....	do.....	do.....	107	19
Feb. 19, 1915.....	do.....	do.....	20	1
Dec. 18, 1914.....	do.....	<i>Hordeum vulgare</i>	31	0
Dec. 24, 1914.....	do.....	do.....	21	2
Dec. 29, 1914.....	do.....	do.....	31	11
Jan. 26, 1915.....	do.....	do.....	48	3
Feb. 1, 1915.....	do.....	do.....	23	8
Dec. 18, 1914.....	do.....	<i>Secale cereale</i>	11	0
Dec. 24, 1914.....	do.....	do.....	14	0
Dec. 29, 1914.....	do.....	do.....	38	1
Feb. 1, 1915.....	do.....	do.....	39	1
Feb. 19, 1915.....	do.....	do.....	41	6
Apr. 3, 1915.....	do.....	<i>Avena fatua</i>	7	2
Apr. 11, 1915.....	do.....	do.....	10	2
Apr. 3, 1915.....	do.....	<i>Avena elatior</i>	20	3
May 29, 1915.....	do.....	<i>Bromus tectorum</i>	23	2
Jan. 12, 1915.....	do.....	<i>Dactylis glomerata</i>	55	37
May 14, 1915.....	do.....	<i>Elymus virginicus</i>	40	1
Apr. 11, 1915.....	do.....	<i>Lolium italicum</i>	21	0
May 29, 1915.....	do.....	do.....	32	3
Do.....	do.....	<i>Lolium perenne</i>	15	4
Do.....	do.....	do.....	40	4
Mar. 3, 1915.....	<i>Hordeum vulgare</i> ^a	<i>Phleum pratense</i>	48	0
Mar. 14, 1915.....	do.....	do.....	40	0
Mar. 7, 1915.....	<i>Avena sativa</i> ^b	do.....	71	0
Apr. 11, 1915.....	<i>Avena fatua</i>	do.....	34	0
Apr. 18, 1915.....	do.....	do.....	15	0
May 2, 1915.....	<i>Phalaris canariensis</i> ^c	do.....	25	0
Mar. 7, 1915.....	<i>Dactylis glomerata</i>	do.....	40	0
Mar. 14, 1915.....	do.....	do.....	56	0

SUMMARY.

Source of inoculating material.	Plant inoculated.	Result of inoculation. ^d	Source of inoculating material.	Plant inoculated.	Result of inoculation. ^d
<i>Phleum pratense</i> ...	<i>Triticum vulgare</i> ..	$\frac{0}{150}$	<i>Phleum pratense</i> ...	<i>Lolium italicum</i> ...	$\frac{3}{53}$
Do.....	<i>Avena sativa</i>	$\frac{37}{195}$	Do.....	<i>Lolium perenne</i> ...	$\frac{8}{55}$
Do.....	<i>Hordeum vulgare</i> ..	$\frac{24}{134}$	Do.....	<i>Bromus tectorum</i> ..	$\frac{2}{23}$
Do.....	<i>Secale cereale</i>	$\frac{8}{143}$	<i>Hordeum vulgare</i> ...	<i>Phleum pratense</i> ..	$\frac{0}{88}$
Do.....	<i>Avena fatua</i>	$\frac{4}{17}$	<i>Avena sativa</i>	do.....	$\frac{0}{86}$
Do.....	<i>Avena elatior</i>	$\frac{3}{20}$	<i>Avena fatua</i>	do.....	$\frac{0}{34}$
Do.....	<i>Dactylis glomerata</i> ..	$\frac{37}{55}$	<i>Phalaris canariensis</i> ..	do.....	$\frac{0}{25}$
Do.....	<i>Elymus virginicus</i> ..	$\frac{1}{40}$	<i>Dactylis glomerata</i> ..	do.....	$\frac{0}{96}$

^a *Puccinia graminis*, originally from *Hordeum jubatum*; on barley 8 urediniospore "generations."^b *Puccinia graminis*, originally from *Dactylis glomerata*; on oats 9 urediniospore generations.^c *Dactylis glomerata* rust after 13 generations on oats and one generation on *Phalaris canariensis*.^d The denominator gives the total number of leaves inoculated, the numerator the number which developed pustules.

It will thus be seen that the rust from timothy transfers directly to three of the common cereals. Neither Eriksson and Henning (3) nor Johnson (4), as previously mentioned, were able to obtain successful infection on barley as a result of direct transfer from timothy. However, the writers were able to infect some plants in four of the five series of inoculations. The percentage of infections on barley is nearly as great as that on oats and is greater than that on rye. The rust transferred very readily to *Dactylis glomerata* and fairly well to both *Avena elatior* (*Arrhenatherum elatius*) and *Avena fatua*. It also transferred to *Lolium perenne*, *Lolium italicum*, and *Bromus tectorum*. One extremely small pustule developed on *Elymus virginicus*.

The vigor of infection varied greatly on different hosts. In addition to the inoculations indicated in Table I, many inoculations were made on timothy. These nearly always resulted in a 100 per cent infection. The incubation period on timothy was 7 to 8 days, while on barley it was 10 to 12 days. It was clearly evident that barley was an uncongenial host; fairly large dead areas were frequently formed without subsequent development of pustules, and all pustules, when they did develop, were extremely small. Most of the pustules were less than 1 mm. in diameter, being mere dots in some cases. However, others were somewhat larger, some attaining a diameter of over 1 mm. On oats the pustules were larger, the rust developing in a fairly normal manner. The pustules on rye were fairly small, but there was not such a distinct tendency to produce flecks as there was on barley. The infection on *Avena elatior*, *Avena fatua*, *Lolium perenne*, and *Lolium italicum* was moderate, while that on *Dactylis glomerata* was very severe, nearly as severe as that on timothy. On *Bromus tectorum* the pustules were extremely small.

Although the rust transferred fairly readily from timothy to both barley and oats, no infection was obtained on timothy as a result of inoculations with *Puccinia graminis hordei* and *Puccinia graminis avenae*. Less than 100 inoculations were made with *Puccinia graminis hordei*; in no case, however, was there any indication of successful infection. The transfer is entirely possible; more inoculations will therefore be made. Timothy was inoculated directly with *Puccinia graminis avenae*, but no infection resulted from any of 86 trials. No better results were obtained by transferring first to *Avena fatua*, *Phalaris canariensis*, or *Dactylis glomerata*. None of these forms, therefore, acted as a bridging form between oats and timothy. It is possible that such bridging forms may exist, although the possibility has not yet been demonstrated. Carleton (1, p. 62) reported successful infection of *Puccinia graminis avenae* on *Phleum asperum*. It is possible that this form might act as a bridging species, but the writers have not yet had opportunity to determine this.

EXPERIMENTS WITH BRIDGING HOSTS

Johnson (4, p. 10) found that by using *Avena sativa* as a bridging host the timothy rust could be transferred to *Hordeum vulgare*; by using *Festuca elatior* it could be transferred to *Hordeum vulgare* and to *Triticum vulgare*; by using *Dactylis glomerata* it could be transferred to *Triticum vulgare*. Since the writers were able to infect barley directly, but not wheat, without the bridging hosts, an attempt was made to determine whether or not, with the strain of rust employed, it would be possible to make transfers to wheat after using *Dactylis glomerata* as a bridging form and whether or not the rust would transfer to barley more readily under the same conditions. Transfers were made from timothy to *Dactylis glomerata*, and heavy infection was obtained. Two series of inoculations were then made with spores from *Dactylis glomerata* to wheat, oats, barley, rye, and timothy. The results were as follows: Wheat, $\frac{0}{19}$; oats, $\frac{2}{88}$; barley, $\frac{8}{36}$; rye, $\frac{2}{32}$; timothy, $\frac{1}{26}$. When oats was used as a bridging host, approximately the same percentage of infections resulted as when the rust was transferred directly from timothy. The writers were, therefore, unable to increase the infection capabilities of the rust by means of first transferring to *Dactylis glomerata* or oats. Neither was the vigor of infection appreciably greater on barley and oats after using bridging species. It is possible that by confining the rust for a long series of generations on a bridging host definite results might be obtained. Such experiments are now under way.

The results cited show that different results may be obtained with different strains of rust. That Johnson (4) and Eriksson and Henning (3) worked with different strains seems entirely probable, in view of the fact that neither was able to transfer the rust directly to barley, while the writers experienced no particular difficulty in making such transfer. The possibility of conflicting results may be clearly shown by results which the writers have recently obtained. Timothy rust and stem rust of oats (*Puccinia graminis avenae*) transferred very readily to *Dactylis glomerata*. But the rusts by no means acquired the same capabilities as a result of growing on *Dactylis glomerata*, at least not in a few generations. When the timothy rust on *Dactylis glomerata* was transferred to oats less than 10 per cent of the inoculated leaves became infected; when the rust was transferred to barley very small pustules were produced on about 16 per cent of the inoculated leaves; when it was transferred to rye, small pustules were produced on about 6 per cent of the inoculated leaves; when it was transferred to timothy 95 per cent of the leaves became infected. When, on the other hand, stem-rust of oats (*P. graminis avenae*) on *Dactylis glomerata* was transferred to oats, 100 per cent of the inoculated leaves became very severely affected; inoculations on barley resulted in 7 per cent of infection; inoculations on rye resulted in no infection (in other experiments the writers have been able to infect

rye with *P. graminis avenae*); no infection resulted from inoculations on timothy. The writers also have two strains of *Puccinia graminis*, both of which have been confined to the same variety of barley for nine months. Both attack barley and a number of wild grasses very readily; neither has ever infected oats; one attacks wheat with extreme vigor and infects rye only with difficulty, while the other is almost entirely unable to infect wheat but attacks rye with great vigor.

It seems fairly clear that, as Johnson (4, p. 10) has previously pointed out, timothy rust and *Puccinia graminis avenae* are quite similar. Both rusts transferred to *Dactylis glomerata*, *Avena fatua*, *Avena elatior*, barley, rye, *Lolium perenne*, *Lolium italicum*, *Bromus tectorum*, and *Elymus* spp.; the oats rust to *Elymus robustus* and *Elymus canadensis*; and the timothy rust to *Elymus virginicus*. With the exception of *Avena fatua*, they transferred with somewhat the same degree of readiness.

MORPHOLOGY OF THE SPORES

Morphologically, however, the two rusts are somewhat different, the spores of *Puccinia graminis avenae* being larger. Spores of *Puccinia graminis avenae*, originally from *Dactylis glomerata* and then confined to oats for 14 successive generations, ranged from 19 to 35 μ in length and from 16 to 24 μ in width, the modes falling at about 30 and 19 μ . The spores of timothy rust on timothy ranged from 17 to 31 μ in length and from 14.5 to 23 μ in width, the modes falling at about 26 and 18 μ . After one generation on *Dactylis glomerata*, the timothy-rust spores ranged from 17 to 32 μ in length, and from 13.5 to 23.2 μ in width, while the modes fell at about 25.5 and 19.5 μ . At least 100 spores from different pustules were measured. Measurements were also made of spores produced after the rust had been one generation on other hosts, including oats, rye, barley, *Lolium perenne*, and *Avena fatua*; but no distinct and consistent differences were apparent, with the exception of the spores produced on barley. These were smaller than those produced on any other host, ranging from 18.5 to 28.3 μ in length and from 13 to 20 μ in width. The modes were at about 23 and 17 μ . Whether or not greater variations would occur if the rust were confined to the different hosts for longer periods of time is not yet known. Experiments have been begun to determine the effect of different hosts on the morphology of the spores.

SUMMARY

(1) Timothy rust was transferred successfully directly from timothy to *Avena sativa*, *Hordeum vulgare*, *Secale cereale*, *Avena fatua*, *Avena elatior*, *Dactylis glomerata*, *Elymus virginicus*, *Lolium italicum*, *Lolium perenne*, and *Bromus tectorum*.

(2) Attempts to increase the infection capabilities of the rust by the use of bridging hosts for short periods of time were unsuccessful.

(3) The infection capabilities of timothy rust are quite similar to those of *Puccinia graminis avenae*.

(4) Attempts to infect timothy with *Puccinia graminis avenae* and *Puccinia graminis hordei* were unsuccessful.

(5) The morphology of the spores of timothy rust on different hosts varies slightly; spores produced on barley were considerably smaller than those produced on more congenial hosts.

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EXPERIMENTS IN THE USE OF CURRENT METERS IN IRRIGATION CANALS

By S. T. HARDING,

Irrigation Engineer, Office of Public Roads and Rural Engineering

INTRODUCTION

Comparisons of the relative accuracy of measurements made in irrigation canals with current meters using different methods are made in the following discussion. In connection with field experiments made on the flow in various types of canals in order to determine the value of the coefficient n of Kutter's formula,¹ detail current-meter gagings were necessary. These detail gagings and other observations made at the same time have been used to compare the results obtained by the standard two-point, single-point, and integration methods, as well as by floats and various selected points of measurement. Much experience is now available in regard to the various methods of current-meter observations used in natural channels. The results given here apply to the more regular artificial channels used in irrigation for which there are fewer available data.

In the experiments referred to, the current-meter readings were carefully taken at from 12 to 20 points horizontally across the canal section, from four to six readings being made at each point. These detail or multiple-point observations were plotted, and the mean velocity at the different points observed was determined from the vertical velocity curves drawn through the plotted observations. The points across the canal at which observations were made are referred to in the following discussion as the "verticals." The results secured by the multiple-point reading both in each vertical and for the discharge as a whole have been taken as the correct velocities and discharges in the comparisons made. In canals of the size used in most of these experiments determinations of

¹ Ganguillet, E., and Kutter, W. R. General Formula for the Uniform Flow of Water in Rivers and Other Channels; translated from the German, with ... additions ... by Rudolph Hering and J. C. Trautwine. ed. 2, 240 p., pl. New York, 1893.

the discharge by other methods than by the use of the current meter would not have been practicable. Greater detail regarding the methods used and the experiments in general will be found in a recent publication,¹ which discusses the results of the determinations of the value of n in Kutter's formula. The field work was carried on by various members of the Division of Irrigation Investigations, as stated in the bulletin referred to.

COMPARISONS OF DIFFERENT METHODS OF MEASUREMENT OF VELOCITIES IN THE VERTICALS

There are four principal methods by which the velocities in different verticals are determined with the current meter: The multiple-point method; the mean of the velocities at the 0.2- and 0.8-depth points, called the "two-point method"; the velocity at 0.6 depth, called the "single-point method"; and the vertical-integration method.

As the main purpose of these experiments was the determination of the value of n , it was desired to make the discharge determinations with as great accuracy as possible. The multiple-point method was used, readings being taken usually at six points in each vertical. This was assumed to give the correct discharge and is the discharge used as the basis of the following comparisons.

The multiple-point readings were usually taken at 0.1, 0.2, 0.4, 0.6, 0.8, and 0.9 of the depth. The meter was held from 30 to 60 seconds at each point. From these measurements the discharge by the two-point or the single-point method was computed and compared with the results of the multiple-point method.

When the field measurements were made, in most of the experiments gagings were also made by the vertical-integration method. Generally one or two complete round trips were made with the meter at each vertical, the vertical movement being from 3 to 16 feet per minute. Much care was used to give the meter a uniform vertical velocity so that each portion of the section would be equally represented in the integrated mean. Two complete round trips were usually made, consuming from 40 to 150 seconds, depending on the depth. The meter was generally moved more slowly in the shallower sections in order to give a sufficiently long time for the reading.

In Table I are given the general results for all experiments. These are divided in five different classes of canal sections, although there is no marked variation for the different groups. These include nearly 100 experiments for the two-point and the single-point methods on canals having discharges of from 2 to 2,600 second-feet. Only 55 experiments

¹ Scobey, F. C. The flow of water in irrigation channels. U. S. Dept. Agr. Bul. 194, 68 p., 9 fig., 20 pl. 1915. See also Scobey, F. C. Behavior of cup current meters under conditions not covered by standard ratings. *In Jour. Agr. Research*, v. 2, no. 2, p. 77-83. 1914.

with integration methods are shown, as measurements by this method were not taken in all cases.

TABLE I.—*Variation in discharge in percentage by the two-point, the single-point, and the integration method, compared with the multiple-point method*

Type of canal cross section.	Two-point method.			Single-point method.			Integration method.		
	Number of observations.	Mean difference from multiple-point.	Average variation of a single observation.	Number of observations.	Mean difference from multiple-point.	Average variation of a single observation. 5 per cent correction applied.	Number of observations.	Mean difference from multiple-point.	Average variation of a single observation.
Rectangular flumes....	27	+0.68	1.45	27	+4.90	2.21	17	+1.06	1.36
Concrete-lined trapezoidal sections.....	15	+ .86	1.42	15	+4.21	1.94	4	+ .72	.93
Shallow earth canals, sloping sides.....	13	- .38	1.08	13	+3.11	3.42	9	- .81	2.44
Shallow earth canals, steep sides.....	25	+1.05	1.74	25	+5.02	2.44	13	+ .36	2.15
Earth canals, relatively deep sections.....	16	+1.07	1.70	15	+6.32	3.18	7	+3.06	3.78
Mean of all.....	96	+ .73	1.51	95	+4.80	2.54	55	+ .76	2.07

Table I shows all three methods to give an average discharge greater than the multiple-point gaging. For the two-point and integration methods this is not large, being about three-fourths of 1 per cent for both of these methods. For the single-point method the average error is +4.80 per cent. This is large enough to warrant a correction factor, so that all further comparisons with this method are based on a correction of -5 per cent made to the discharge secured by the single-point method.

Besides the average error of the series of experiments, the probable or average variation of a single observation is also given. While the mean difference of the two-point and integration from the multiple-point method is the same, the single measurements show a somewhat greater average variation for the integration than for the two-point method. If the results of the single-point observations are reduced by 5 per cent, the corrected results have an average variation but little in excess of the other methods. These results may be expressed by saying that with the two-point method a series of observations will give results three-fourths of 1 per cent too high. If no correction is made to the results, single observations will have an average error of 1.5 per cent.

The experiments covered a wide range of discharges and canal types, so that further classifications were made to determine the effect, if any, of differences in the velocity, the depth, or the value of n on the accuracy of the different methods. The results are given in Table II.

TABLE II.—Comparisons of variations in percentage of discharge by two-point, single-point, and integration methods from discharge by multiple-point methods for different velocities, depths, and values of n

COMPARISONS FOR DIFFERENT VELOCITIES

Observation.	Two-point method.			Single-point method (corrected by—5 per cent.).			Integration method.		
	Number of experiments.	Mean difference from multiple-point.	Average variation of a single experiment.	Number of experiments.	Mean difference from multiple-point.	Average variation of a single observation.	Number of experiments.	Mean difference from multiple-point.	Average variation of a single observation.
Velocities in feet per second:									
Less than 1.00.....	5	+1.02	2.56	5	+1.64	3.68	3	+2.60	3.16
1.00 to 1.50.....	18	+ .63	1.59	18	+ .43	2.90	11	+1.62	1.75
1.50 to 2.00.....	12	— .38	1.60	13	+ .24	2.67	10	+1.43	2.80
2.00 to 2.50.....	20	+ .89	1.54	19	— .01	2.66	10	— .02	2.51
2.50 to 3.00.....	14	+1.67	1.68	13	— .25	1.67	10	— .18	1.41
3.00 to 4.00.....	15	+1.11	1.41	15	— .14	1.91	9	+ .50	1.81
Over 4.00.....	12	+ .02	.73	12	—2.66	2.83	2	— .43	.72
Mean.....	96	+ .73	1.51	95	— .20	2.54	55	+ .76	2.07

COMPARISONS FOR DIFFERENT DEPTHS

Mean depth of canal section in feet:									
Less than 1.00.....	14	—0.65	2.06	14	+1.02	3.66	10	+1.98	2.65
1.00 to 1.50.....	18	+ .21	1.23	17	+ .53	1.90	8	+1.46	1.65
1.50 to 2.00.....	15	+1.32	1.73	15	— .03	2.51	7	+ .86	1.49
2.00 to 2.50.....	22	+1.29	1.58	22	— .10	2.82	12	+1.08	2.81
2.50 to 3.00.....	16	+ .97	1.25	16	— .77	1.95	12	— .60	1.84
Over 3.00.....	11	+1.09	1.19	11	— .79	2.44	6	— .26	1.33
Mean.....	96	+ .73	1.51	95	— .20	2.54	55	+ .76	2.07

COMPARISONS FOR DIFFERENT VALUES OF n

Value of n in Kutter's formula:									
Less than 0.013....	13	+0.40	0.76	13	—1.70	2.27	5	+0.90	0.90
0.013 to 0.017.....	18	+ .52	1.42	18	— .32	2.73	11	+ .23	2.25
0.017 to 0.021.....	20	+ .72	1.45	20	— .85	2.16	13	— .52	1.52
0.021 to 0.025.....	13	+ .80	2.17	13	+1.81	2.52	6	+1.87	2.91
0.025 to 0.029.....	11	+ .53	.88	11	— .41	2.56	5	—1.30	1.55
Over 0.029.....	11	+ .66	1.72	10	— .41	3.00	5	+4.35	4.35
Mean.....	86	+ .61	1.41	85	— .35	2.50	45	+ .59	2.14

The two-point method appears to give results equally accurate for all velocities, depths, and values of n , the variations which occur not being seemingly dependent on any of these three factors. The probable error of a single observation is generally less for the large velocities and

depths, which is also true of the other methods. This is to be expected, as the smaller velocities and depths usually occurred in canals of small discharge, where the general conditions for the use of the current meter are not so favorable. The accuracy does not appear to be affected by the character of the channel or value of n .

There is some indication that the correction to be used with the single-point method should be greater than 5 per cent for low velocities and less for the higher ones. This tendency is not marked, however, and it is doubtful if it is sufficient in amount or that it is sufficiently proved by these results to warrant the use of different corrections; also the correction seems to vary with the depth in a similar way.

The integration method seems to give the closest average results for velocities from 2 to 3 feet. It also appears to be more accurate for the greater depths. This latter result is to be expected. In the use of the integration method the velocity in from 0.2 to 0.3 foot in depth must be either missed entirely or imperfectly determined both at the bottom and at the water surface. The velocity at the bottom is lower than the average. Therefore the measurements in the remaining portions of the depth would give results above the actual average velocity. As the proportion of the depth for which velocities are undetermined is larger in the shallow canals, the proportionate error would be greater.

Another method sometimes used is that known as the three-point method, in which the velocity is measured at 0.2, 0.6, and 0.8 of the depth. This is more usually computed by giving the velocity at 0.6 depth equal weight with the mean of the 0.2 and 0.8 depth velocities. As Table I shows the single-point method to be less accurate than the two-point, there is no apparent advantage in the three-point method over the two-point. In sections where the two-point method gave results too low and the single-point too high, their combination might increase the accuracy over that secured by the two-point method alone. Where both were of the same sign, the use of the three-point method would give less accurate results than the two-point alone. The two-point and single-point methods gave results having opposing signs on less than one-third of the total number of experiments, so that the three-point would seem to have little advantage over the two-point method.

To definitely determine the relative accuracy of the three-point method, the discharge of each experiment was computed, using both the method by which the velocity at 0.6 depth is averaged with the mean of the velocities at the 0.2 and 0.8 depths, and also the method by which the velocities at the three points are given equal weight. This latter method would seem to be the more logical, as it has been shown that the two-point, or 0.2 and 0.8 depth method, gives results more accurate than the 0.6 point alone, so that in the use of the three points it would be preferable to reduce the weight given to the velocity at 0.6 depth.

The results of this comparison are given in Table III, which shows that the second method of computation gives the more accurate results. In no class of canal section does either three-point method give as accurate average results as the 0.2 and 0.8 depth method alone. In the individual experiments in one-seventh of the total number the $\frac{0.2+0.8+2 \times 0.6}{4}$ method gave more accurate results than the 0.2 and 0.8 depth alone. In one-fifth of the total number the $\frac{0.2+0.8+0.6}{3}$ method gave results more accurate than the 0.2 and 0.8 depth alone. These were for gagings in which the errors of the 0.2 and 0.8 depth method were of different sign from those of the 0.6 method, so that their combination reduced the actual error. These cases were generally for canals of irregular section and flow, and indicate that for unfavorable conditions of current-meter work the three-point method may be preferable to the two-point, but that for usual conditions the two-point alone is preferable. However, under unfavorable conditions of irregular velocity and cross section only detail multiple-point observations can be depended upon for accurate results. The $\frac{0.2+0.8+0.6}{3}$ method is always preferable for computation of the results to the $\frac{0.2+0.8+2 \times 0.6}{4}$ method.

TABLE III.—Variation in discharge in percentage by the three-point method compared with the multiple-point method

Type of canal cross section.	Number of observations.	Average variation from multiple-point method.	
		Giving velocity at 0.6 depth equal weight with mean of velocities at 0.2 and 0.8 depths. Mean velocity = $\frac{0.2+0.8+2 \times 0.6}{4}$	Giving velocities at 0.2, 0.6 and 0.8 depths, equal weight. Mean velocity = $\frac{0.2+0.6+0.8}{3}$
Rectangular flumes.....	21	+2.5	+1.8
Concrete-lined trapezoidal sections....	15	+2.7	+2.0
Shallow earth canals, sloping sides.....	11	+1.7	+1.3
Shallow earth canals, steep sides.....	21	+2.5	+2.0
Earth canals, relatively deep sections..	14	+3.5	+2.7
Mean of all.....	82	+2.6	+2.0

MEASUREMENTS WITH SURFACE FLOATS

In many experiments measurements with surface floats were made in order to secure data from which the proper coefficients for use with such measurements could be derived. It is often convenient to make such approximate measurements by timing floats over a known length of canal and applying some coefficient to the product of the velocity so

secured and the cross section of the canal in order to give the discharge. In such measurements there are two principal sources of error: (1) The cross-sectional area is difficult to obtain except in flumes or lined canals of uniform cross sections and (2) mistakes may be made in choosing a coefficient to be used in reducing the maximum surface velocities as obtained from the floats to the mean for the whole canal.

The following results relate to the proper coefficient to be used to reduce surface-float velocities to the mean velocity for the whole cross section. The average errors discussed are those arising from the determinations of float velocities and the choice of coefficients and do not include errors in determining the canal cross sections. For the other purposes of these experiments the areas of the canal sections were carefully determined. In the usual field use of float methods there may be a considerable error introduced due to errors in the approximate determinations of canal cross sections of variable dimensions, which would give larger probable errors for the discharge than would result from the probable error due to the choice of the coefficient to use with the velocity of the float alone.

Various formulas have been derived for the relation of the surface velocity to the mean velocity. These have been derived both for the relation of the surface velocity to the mean velocity in any single vertical in the section and for the relation of the maximum surface velocity to the mean velocity of the whole channel. Ganguillet and Kutter¹ give a formula, deduced by Bazin, in which the ratio of the maximum to mean velocities in a channel are made to vary with

$$\sqrt{\frac{RS}{V^2}}$$

As this term is equal to the C in Chezy's formula, a table is given for the value of the ratio for different values of C . In this formula Kutter substitutes the values of n and R from his general formula and gives a table for the values of the ratio of mean to maximum velocity, depending on R and n . The formula derived by Bazin, which forms the basis of this table, was based on 61 series of gagings.

In the canal experiments discussed in this paper in which float measurements were made several small floats would be started simultaneously at scattered points in the portion of the channel having the highest velocities. The time of the most rapid float was used to compute the maximum surface velocity. This gives lower coefficients than would be obtained by the use of the average of all floats. Small floats such as twigs or chips were used which would have both a small submergence and a small exposed surface above the water. It was found that there was little difference in the velocities of the floats thrown into the main threads of the canals unless some became caught in noticeable side

¹ Ganguillet, E., and Kutter, W. R. Op. cit.

eddies. The floats were generally timed over the 500 to 1,000 feet of canal used in the value-of- n experiment.

The value of the coefficient for each experiment was compared with the coefficient given in Kutter's table for the same value of R and n . For all measurements the coefficients differed by an average of 0.06. The mean of all observations was 0.013 lower than Kutter's. This is not an unreasonable variation when it is remembered that at best the method is only an approximate one.

The selection of the coefficient based on the value of R and n is not, however, a convenient one for field use. The determination of the canal cross section, except for flumes and lined sections, will be approximate and the determination of the value of R even more uncertain. A variation of the coefficient with the water area would be the most convenient for field use. A field measurement involves the determination of the mean cross section of the canal and the velocity of the float. If the selection of the proper coefficient is based on the cross section and an estimated value of n no additional measurements or computations are required in order to select the proper coefficients. The experiments give evidence that the coefficient varies with the character of the wetted surface, so that some knowledge of the value of n is required.

In order to determine the value of the coefficients for different conditions, the results of each measurement were plotted with the value of the coefficient and the cross-section area as coordinates. A series of curves for the different values of n were fitted to these plotted observations and adjusted until they gave results equaling, on the average, the results of the actual field determinations. From these curves the values of the coefficients given in Table IV were secured. No attempt was made to derive an equation for the variation in the value of the coefficient, graphical methods being used throughout.

TABLE IV.—Coefficients to be applied to velocities of floats to obtain mean velocity in canals

Area of water cross section.	Value of n in Kutter's formula.									
	0.012	0.014	0.016	0.018	0.020	0.022	0.024	0.026	0.028	0.030
<i>Square feet.</i>										
2	0.85	0.80	0.76	0.73	0.70	0.67	0.65	0.63	0.61	0.60
486	.81	.77	.74	.71	.68	.66	.64	.62	.61
687	.82	.78	.74	.71	.68	.66	.64	.63	.62
888	.83	.79	.75	.72	.69	.67	.65	.63	.62
1088	.83	.79	.76	.73	.70	.68	.65	.64	.63
1589	.84	.80	.77	.74	.71	.69	.66	.65	.64
2090	.85	.81	.78	.75	.72	.70	.67	.66	.65
2591	.86	.82	.78	.75	.73	.71	.68	.66	.65
3091	.86	.82	.79	.76	.73	.71	.68	.67	.65
3591	.86	.82	.79	.76	.73	.71	.69	.67	.66
4091	.86	.82	.79	.76	.73	.71	.69	.67	.66
5091	.86	.82	.79	.76	.73	.71	.69	.67	.66
Over 5091	.86	.82	.79	.76	.73	.71	.69	.67	.66

From these experiments it appears that the coefficient is constant for different values of n for cross-section areas over about 35 square feet. The rate of variation of the coefficient is greatest for the smaller channels. The observations for cross-sectional areas over 100 square feet were too few in number to give dependable averages for canals of larger size, but both these results and Bazin's formula indicate that the coefficient is practically constant for such larger cross sections.

Similar curves were also obtained based on the value of the coefficient and the discharges. These were similar in form and indicate that the velocity within the limits of the experiments did not materially affect the ratio of maximum surface to mean velocity. These values are not given, as the coefficients based on canal areas are more convenient to use.

The results were further classified by the shape of the channel. Apparently the coefficient does not vary with the form of cross section, as the coefficient from the curves agrees fairly well with the observations when the proper values of n are used, whether the canal is rectangular or irregular or whether the section is deep or shallow relative to its width.

The average variation of the observed coefficients from the curves was 0.045. The average of all observations agreed with the curves, the plus variations equaling those of minus sign. Expressed as a percentage, the average variation was 6. For any single observation the observed value of the velocity coefficient is as likely to differ from the mean curve by less than 0.045 as it is to differ by more than this amount. For the average values of the coefficient this amounts to a variation of 6 per cent. In 17 of the 92 experiments, or 18.5 per cent of the total number, the observed value differed by over 10 per cent from the curves.

The more usual practice where such methods of measurements of velocities by floats are made is to use some general value of the coefficient, usually 0.80 or 0.85. These experiments, as well as the observations given by Kutter, clearly indicate that the coefficient varies quite materially for different-sized canals and for different values of n . These results give values for the coefficients which are less than 0.80 for all values of n over 0.016, becoming as low as 0.60 for small canals having high values of n .

The value of n for any given canal is, of course, uncertain to some extent. The coefficient varies most rapidly with the lower values of n . An error of 0.002 in selecting the value of n makes a difference of 5 per cent in the value of the correct coefficient to be used for low values of n , and less than 2 per cent for the higher values.

The coefficients to be used should be selected from the cross-section area and the value of n . The character of the canals corresponding to the different values of n given in Table IV can be secured from the general list following:

Values of n

- 0.012. Straight wood flume in good condition; clean concrete lining having very smooth finish; no moss or gravel.
- 0.014. Ordinary straight wood flumes, little rock or sand; unplastered concrete lining; no moss or gravel.
- 0.016. Worn wood flumes containing growths or sand and gravel; average concrete linings, irregular finish, moss growths or gravels; best earth canals, uniform silted and clean sections.
- 0.018. Very poor wood flumes; rough concrete with covering of moss or gravel; very good earth canals; uniform section, silted, free from gravel and moss.
- 0.020. Concrete in poor condition, much moss and gravel; better than average earth sections without growths and fairly regular sections.
- 0.022. Earth sections, generally free from moss or gravel.
- 0.024. Average earth canals, fairly clean and regular, some gravel and vegetation.
- 0.026. Earth canal; gravel and some cobbles, some moss, irregularities in cross section; masonry-lined canals.
- 0.028. Canals with some cobbles; moss and other unfavorable conditions.
- 0.030. Earth canals, much moss or weeds, irregular section, gravel or cobbles; fairly smooth rock cuts.

It is preferable to make float measurements on straight portions of canals. If it is necessary to use a length containing curves, a coefficient should be selected for a value of n about 0.002 higher than would otherwise be used.

These experiments give data both on the most probable coefficients to be used in float measurements and also on the limitations of accuracy to be expected. Such measurements are often desirable for quick approximate determinations. The most rapid of several floats should be used and the proper coefficient selected to fit the conditions. The error from the float determinations should not often exceed 10 per cent, although error in estimating the cross-sectional area may result in much larger errors in the resulting discharge for earth canals. In flumes or section of regular forms the error in determining the water area should not be large.

EFFECT ON ACCURACY OF CURRENT-METER GAGINGS FROM THE USE OF DIFFERENT NUMBERS OF OBSERVATIONS ACROSS THE WIDTH OF CANALS

The number of verticals across a gaging station at which velocity measurements should be made is a question on which there has been much difference of opinion.

In the sections of irrigation canals at which current-meter gagings are generally made, the cross section is more regular than in the usual stream gaging station, so that usually fewer measurements should be required. In the experiments discussed, measurements were made in from 13 to 20 verticals with a minimum distance apart of the verticals of 0.5 foot on the smaller canals. These measurements are more than are usual in general field practice. The results obtained were compared with the

discharge which would have been obtained had a less number of verticals been measured. The different types of canal sections were grouped into general classes. For each gaging, discharges using only every other vertical measured were computed and also using only every fourth vertical. Two computations of each gaging using the two sets of alternate verticals were made, and also two sets for every fourth vertical. These results were then compared with the discharge obtained by the use of all the verticals measured, in order to determine the probable errors to be expected when fewer verticals were used. The average number of verticals observed in the experiments was 16; the number in the comparisons averages 8 and 4. In general field current-meter work, if only 8 or 4 verticals had been measured, the ones used might have been located in the cross section differently from the arbitrary method used in this computation, so that the selection of alternate verticals as used should give errors larger rather than smaller than are to be expected. The results of this comparison are given in Table V.

TABLE V.—*Effect on the accuracy of current-meter gagings of varying numbers of verticals*

Type of canal.	Number of detail gagings made.	Average number of verticals in detail gagings.	Comparisons using one-half of observed verticals. Variation (per cent).		Comparisons using one-fourth of observed verticals. Variation (per cent).	
			Average.	Minimum and maximum.	Average.	Minimum and maximum.
Flumes, vertical sides.....	23	15	0.9	+0.05 to -3.82	2.9	0 to -7.50
Concrete-lined canals; steeply sloping sides.....	11	14	.9	-.04 to -2.95	2.9	-1.08 to -5.85
Concrete-lined canals; wide and flatly sloping sides.....	6	17	1.4	-.37 to -3.22	3.8	-.70 to -6.52
Average earth canals, sloping sides.	18	16	2.9	+.1 to -8.3	9.2	-1.5 to -17.6
Average earth canals, steep sides..	21	16	2.5	+.1 to -7.3	9.0	-.4 to -21.1
Earth canals, relatively deep sections.....	10	16	2.7	-.5 to -5.5	7.7	-.6 to -19.4
Mean of all...	89	16	1.9	6.2

Table V gives both the average difference in percentage and the range of variations in single gagings. Occasionally the use of a less number of verticals may give a greater discharge than that obtained from a more detailed gaging, owing to irregularities in the cross section or velocity. Where an average of 4 verticals were used, less than 2 per cent of the observations gave larger discharges than the use of all verticals, so that the average difference is practically equal to the mean error. Where 8 verticals were used for all observations, one in each seven measurements

gave results larger than the use of all verticals. Except for flumes with vertical sides, however, only 7 per cent of the results were larger. In vertical-sided flumes one-third of the results were larger, so that while all experiments on flumes gave an average variation of 0.9 per cent, the mean of all variations was -0.6 per cent.

Table V indicates that in flumes or lined sections such as are usually used for canal-rating sections, the observation of velocities in from 12 to 20 verticals will give an increased accuracy of about 1 per cent over the results obtained with from 6 to 10 verticals, and about 3 per cent greater than with from 3 to 5 verticals. Under the most favorable conditions where the rating curve will remain fixed, the measurement of from 12 to 20 verticals, depending on the size of the section, may be warranted. Where the rating may be affected by channel changes during the season or under such conditions as are usually obtainable in the field, measurements based on from 6 to 10 verticals should represent good practice. The use of from 3 to 5 verticals will give results as closely as the rating curves derived can be applied to changing channel conditions in many cases and may be sufficiently close for some purposes. Using 8 verticals, only one-seventh of the results differed by more than 2 per cent; and using 4 verticals, only one-sixth differed by more than 5 per cent.

In the more irregular earth sections larger variations were found. This is to be expected, as in these the velocity and depths both change more rapidly near the sides than in the case of flumes. The use of an average of 8 verticals in earth sections gives results of similar accuracy to those obtained with only one-half as many verticals in flumes and lined sections. The use of an average of only 4 verticals gives results with average differences of nearly 9 per cent, and the variations of single experiments are much greater. It would appear that to obtain equal accuracy in gagings in earth sections with those secured in flumes about twice as many verticals should be observed. The number used will depend on the accuracy desired and the size of the canal. Less than from 6 to 8 verticals can not be recommended, and probably 8 to 12 would represent good practice. For more accurate work from 15 to 20 may be used, although where great accuracy is desired the measurements should be made in regular rating sections. Using 8 verticals, only one-tenth of the experiments differed by more than 5 per cent; using 4 verticals, one-third of the results differed by over 10 per cent.

A comparison of these results with those given for the different methods of observation of the velocity in the verticals can be made to determine the relative advantages of using either more verticals or taking more points in each vertical. The use of the 0.2- and 0.8-point method gave results averaging 0.7 per cent too high. The use of an average of 8 verticals in flumes and lined sections gave an average of 0.6 per cent too small. The use of 8 verticals obtained with the 0.2 and 0.8 method would tend to balance these errors, and in many cases might give as

accurate results as the more detailed observations. The use of the 0.6-point method gave results averaging 4.8 per cent too high, and the use of from 3 to 5 verticals in flumes and lined sections gives an average of 3 per cent too small. Apparently where few verticals are to be observed, the use of the 0.6-point method may be preferable, as the errors will tend to balance. This may be expressed by saying that about the same relative detail should be used in measuring the velocities in the verticals that is used in the number of verticals observed.

The results are obtained by using the verticals taken in the detailed measurements and selecting every alternate or every fourth vertical and computing the discharge that would have been obtained had only these verticals been observed. It is possible that gagings where the lower numbers of verticals were to be observed could be made to give closer results by using some means for the selection of the location in the canal section at which the verticals should be taken. It has been previously shown that the use of velocity measurements at the 0.2- and 0.8-depth points will give very nearly the same results as measurements at 6 or more points in the vertical and that a single observation at 0.6 gives results within 5 per cent of being correct.

If one or two points can be found in the vertical velocity curves the velocities of which can be used to determine the average velocity of the whole vertical, it would seem probable that perhaps 2 verticals on the horizontal velocity curve could be found which could be used to give the average velocity in the whole cross section. Such points, or index verticals, as they may be called, would be useful in the rougher measurements often needed in canal operation, and information as to the relative accuracy of such methods should be of value.

Two such selected verticals may be used to determine the discharge in two ways. In one the velocities only might be used and the cross-section area more carefully determined, if not known from previous observation. In the other the observed verticals may be used to obtain not only the mean velocity but also the depths at these verticals, and the width of the section may be used to determine the cross-sectional area.

The use of such index-vertical methods is, of course, most applicable to canal sections such as flumes which have practically uniform depths, as the error in determining the cross section is largely eliminated.

The measurements were examined to see whether such index verticals could be found. The horizontal velocities and cross sections were plotted on a sufficiently large scale so that the velocity and depth at any point could be read from the curves. Such index verticals would be most easily used if their distance from the sides is some definite proportion of the water-surface width. Verticals located at different points were tried. The different types of canal cross sections are discussed separately. The general results are given in Table VI.

TABLE VI.—Discharge and velocity of various types of canals by measurements of two selected verticals

Observations.	Number of observations.	Difference from correct discharge (per cent).			
		Average error of all observations.	Average variation of a single observation.	Extent of variations.	
				Plus.	Minus.
Total discharge in flumes with vertical sides:					
For points one-fifth from sides.	22	+ 1.7	2.1	+ 4.5	- 1.4
For points one-sixth from sides.		- 1.4	2.6	+ 5.1	- 6.5
Velocities only:					
Concrete-lined canal, steep side slopes—					
For points one-fifth from sides.	10	- 2.3	2.7	+ 2.0	- 5.7
For points one-fourth from sides.		+ 1.3	3.1	+ 6.1	- 3.7
Concrete-lined canals, wide and flatly sloping sides—					
For points one-fifth from sides.	6	- 1.0	1.0	+ 1.6	- 5.3
For points one-fourth from sides.		+ 1.4	1.8	+ 3.6	- 1.2
Average earth canals, sloping sides—					
For points one-fifth from sides.	15	- 2.3	5.8	+ 9.0	-13.6
For points one-fourth from sides.		+ 4.3	5.2	+15.4	- 3.8
Average earth canals, steep sides—					
For points one-fifth from sides.	20	- 4.2	5.6	+ 7.2	-17.8
For points one-fourth from sides.		+ 4.9	5.7	+12.1	- 6.7
Earth canals, relatively deep sections—					
For points one-fifth from sides.	8	- 5.7	8.2	+10.0	-19.6
For points one-fourth from sides.		+ 2.6	5.3	- 9.4	+13.8
Total discharges, points one-fifth from sides:					
Average earth canals, sloping sides.	15	+ 1.1	5.0	+14.6	- 8.2
Average earth canals, steep sides.	20	+ 3.3	6.0	+14.2	-10.4
Earth canals, relatively deep.	8	+ 2.0	7.0	+14.1	-12.1

For vertical-sided flumes 22 gagings were available. The depths varied from 0.7 to 4.4 feet, the widths from 2 to 17.7 feet, and the discharges from 2 to 400 second-feet. The velocities and depths at points at a distance of one-fifth and one-sixth of the width from the sides were used to obtain discharges which were then compared with those obtained by the complete gaging. In such flumes with vertical sides the depths are practically uniform, and the use of the depth at only two points would cause little error in the resulting area. These results show that the two points whose mean velocities will equal that of the whole cross section lie generally between one-sixth and one-fifth of the width from the sides and that the error in using such index velocities at either proportion of the width averages about 2 per cent and does not exceed 5 per cent, except in a few cases.

For concrete-lined canals the canal section is uniform, and the cross-sectional area would be known for any depth. In such canals the discharge can be obtained from determinations of velocity and known areas for given depths. The comparisons given in Table VI are based on

velocity alone. The lined sections were subdivided into two classes: Those with relatively steep sides and those following the flatter slopes more usual to earth canals. There is no marked difference in the results of the two types. These measurements indicate, as was to be expected, that the points of mean velocity are farther from the water edges in sections with sloping sides than in the vertical-sided flumes and occur between one-fifth and one-fourth of the width of the water surface from the edges. The average and maximum errors are not large.

For earth canals results are given for both velocities and for total discharges. The results for such sections are more variable. The velocity at from one-fifth to one-fourth of the width from the edges will average to give results close to the actual velocity for the whole section, but individual gagings may vary from the mean by over 15 per cent. The results for the total discharge are more consistent than those for velocity alone. The error in the cross-sectional area, due to using the two measurements of depth to give the mean depths, tends to balance some of the errors in velocity. For all measurements the determination of the depth and velocity at points one-fifth of the width of the water surface from the sides gives average results from 1 to 3 per cent too high. Any single gaging will average to give errors of 5 to 7, and they may be as high as 15 per cent.

These results indicate that under favorable conditions two index verticals can be found in canals, the velocity at which will agree with the average for the whole cross section. These points are from one-fifth to one-sixth of the width of the canal from the sides in sections with vertical sides and from one-fourth to one-fifth for other types. In sections with vertical sides, such as flumes, and in earth sections the depths at these index verticals will also be quite close to the average depth in the whole section, so that the index points can be used also to determine the total discharge. In definite sections with sloping sides, such as concrete-lined canals, it is preferable to use known relations of depth and area and use the index points for the determination of velocities only.

Such short-cut methods would not generally be desirable at permanent rating stations. They might be useful for approximate measurements where time was an important factor, or as checks on the division of water in canal at large turnouts. Such gagings could be made of the canal above and below, and also of the turnout. Where other means of measuring or controlling the device are not available, such rapid methods might be of value.

SUMMARY

Comparisons of various methods of current-meter gaging of irrigation canals are made with measurements in which the velocities at from 70 to 120 points were taken. Canals of various types of cross section having discharges of from 2 to 2,600 second-feet and velocities of from 0.5 to 8.0 feet per second were included.

In 96 measurements the 0.2- and 0.8-depth, or two-point, method gave results averaging 0.73 per cent too high, and the 0.6-depth, or single-point, method gave results 4.80 per cent too high. The average variation for a single measurement was 1.5 per cent for the two-point method. If the results for the single-point method are corrected by -5 per cent, the average variation of a single observation is 2.5 per cent.

In 55 measurements the vertical integration method gave results averaging 0.76 per cent too high, and an average variation for a single observation of 2.07 per cent. The use of three-point methods gave errors greater than the two-point method alone.

There were no marked variations of the accuracy of any of these three methods due to difference in velocity, depth, or value of n in Kutter's formula.

In 92 measurements to determine the coefficient to be used to reduce the maximum surface velocity as measured by small floats to the mean for the entire cross section, the coefficient was found to vary with the value of n in Kutter's formula and the size of the canal. For water cross sections of over about 35 square feet the coefficient remains constant for any given value of n . A table is given for the coefficients for the range of conditions covered by the measurements. The coefficient varies from 0.60 to 0.91 for different conditions. The average variation of the coefficient for a single observation from the mean values was about 6 per cent, and in one-fifth of the observations exceeded 10 per cent.

In 89 experiments on the use of observations of varying numbers of verticals across the width of canals, it appears that in uniform cross sections, such as flumes or lined canals, observations in 8 verticals give an average within 1 per cent and in 4 verticals within 3 per cent of the discharge obtained with 16 verticals. In earth canals observations in 8 verticals give an average within 3 per cent and 4 verticals within about 9 per cent. For equivalent accuracy about twice as many verticals should be observed in ordinary earth sections as in uniform lined sections.

It was found that the use of only 2 verticals located from one-fifth to one-sixth of the width of the water surface from the sides of the section in canals with vertical sides such as flumes, gave results within an average of 2.5 per cent. In concrete-lined sections with sloping sides similar results were obtained where the velocities were measured at from one-fifth to one-fourth of the width from the sides, and the areas were secured from the known cross sections.

In earth canals 2 points from one-fifth to one-fourth of the width of the water surface from the sides give velocities varying from the mean of the whole cross section by about 6 per cent. Where the depths at these two points are used to give the average depth, the total discharge is determined with an average error of about 6 per cent. Errors in individual experiments were much higher.

RELATION OF SULPHUR COMPOUNDS TO PLANT NUTRITION

By E. B. HART, *Chemist*, and W. E. TOTTINGHAM, *Assistant Chemist, Department of Agricultural Chemistry, University of Wisconsin*

INTRODUCTION

The four elements, nitrogen, phosphorus, potassium, and calcium, still play the most important rôle in soil treatment. For a number of years, however, other materials which stimulate growth in vegetation have been studied by chemists and agronomists.

The so-called catalytic fertilizers, such as the salts of manganese, have often been shown to increase plant growth. In addition, studies have been made of radium, lithium, sodium, arsenic, barium, copper, and some other elements. While these may stimulate plant growth, their application is not at present regarded as of economic importance. These elements are either not at all necessary for the plant's cycle of growth or, so far as we know, are abundantly supplied in all ordinary soils.

In the case of sulphur the relation appears to be somewhat different. It was pointed out in 1911 by Hart and Peterson (5)¹ that the total sulphur content of the soils examined was low, being approximately equal to the phosphorus content. This work has been confirmed by Shedd (12) for Kentucky soils and by Robinson (11) for the important soil types of the United States. It was further shown by Hart and Peterson (5) that the sulphur content of our common farm crops was considerable, cereal grains containing about half as much sulphur as phosphorus and legume hays sometimes more sulphur than phosphorus, while the Cruciferae, such as cabbage, turnips, etc., may contain two to three times as much sulphur as phosphorus.

It has been urged by Hopkins (6) that the high sulphur content of plants does not represent their needs, but merely shows the superabundance of sulphates in the soil water, with an extraordinary consumption by the plant. This may apply to the stem and roots of plants, but not to the seed. The seeds maintain a fairly constant composition and, as shown by Peterson (9), either contain but traces of sulphates, or more probably none at all. The criticism, then, that a high sulphur content of a plant merely represents a large soil supply can not possibly hold for seeds. It is true that the sulphate sulphur and probably other forms of sulphur in the stems and roots of plants will vary with the soil supply. In these plant parts sulphates may be present where the soil supply is plentiful. The same statement, however, is equally true of phosphates.

¹ Reference is made by number to "Literature cited," p. 249.

Minimum requirements for maximum plant development have never been established for any of the essential elements. In addition, the demands for sulphur will be related to the character of the plant compounds elaborated by the different species of plants, even in the leafy portion. A cabbage crop that absorbs 100 pounds per acre of sulphur trioxid makes use of this material in a different way from a potato crop which absorbs but 11 pounds of sulphur trioxid. In the cabbage, sulphur compounds characteristic of the species are formed in abundance, thus creating a demand for a large sulphur supply. Alfalfa hay, constructed abundantly of protein compounds even in the stem and leaf, will demand and contain more sulphur than the low-nitrogen-containing residual straws of cereals. In either of the above cases used for illustration—namely, cabbage and alfalfa—it has been found that 30 to 50 per cent of the total sulphur may be present as sulphate sulphur. Nevertheless, this makes the total organic sulphur in an acre's growth of these crops very considerable—about 30 and 50 pounds of sulphur trioxid, respectively. In this connection let us again mention the fact that the annual rainfall will carry to an acre not more than 17 to 20 pounds of sulphur trioxid, while the loss by drainage may equal and even exceed this quantity. While we have no knowledge as to whether the excess of sulphates absorbed by the plant is of physiological importance, it is, nevertheless, clear that a supply of sulphur in this form in the plant indicates that the plant has not been limited in the elaboration of organic compounds for which sulphur is necessary. In fact, we suggest that information as to whether sulphur is a limiting factor for plant growth in any soil may probably be obtained by testing for the presence of sulphates in the plants grown on that soil. Their presence would indicate that there was a sufficient supply for all constructive purposes in which sulphur is involved.

From the facts presented on crop demand and soil supply we seem perfectly justified in including sulphur with nitrogen and phosphorus in the first group of essential elements which are limited in quantity in our common soils and in constant and relatively large demand by crops. On the same basis, potassium, calcium, and magnesium fall into a second group, while iron, constituting the third group, represents an element usually in abundance in soils and utilized in but small quantities by farm crops. Consequently, on the basis of total analysis and mathematics, sulphur should be of equal importance with phosphorus. Here, however, is where very probably total analysis and mathematics will not find complete justification for their use as the sole instruments in measuring permanent soil production. In collaboration with Prof. Fred (3), the senior author has pointed out the very great difference in the effect of phosphates and sulphates on important biochemical processes in the soil. In these studies it has been shown that soluble phosphates increase enormously the number of soil organ-

isms and the rate of ammonification and destruction of organic matter, while the sulphates activate but slightly in these directions. The processes mentioned are admitted to be of great importance to the plant's nutrition and environment, involving, as they must, not only a more rapid formation of readily soluble compounds of nitrogen and a possible destruction of harmful organic materials, but a greater saturation of the soil moisture with carbon dioxid, resulting in increased solution of mineral materials necessary for rapid growth.

While from the application of analytical chemistry and mathematics we should be led to give equal importance to phosphorus and sulphur in plant production, from their relation to important soil biochemical processes we must certainly ascribe to phosphorus the more important rôle. It has been demonstrated beyond question in certain phases of fermentology that cellular and enzymic activities are markedly increased by the presence of soluble phosphates. Harden and Young (4) have shown that the activation of the yeast cell or its zymase is greatly accelerated by the presence of these substances, and we now know that such activation by phosphates is not confined to the yeast plant but may also extend to the soil flora.

Consequently, in the case of phosphorus we have at least two factors operating to make it important in the soils—supply and physiological action; while in the case of sulphur the more important rôle will be merely as a source of supply. This, however, may not always be its only function, as will be shown later, where in the case of red clover it appears to have rather specific effects on root development; but besides such specific effects it appears at present that sulphur as sulphate in the soil serves essentially as the source of needed sulphur. It, therefore, in our judgment becomes important to accumulate information as to which agricultural plants will be affected by an increased concentration of sulphates in the soil water.

For some time sulphur in its elemental form has been used in the control of certain plant diseases. Incidental to this work there has accumulated much contradictory evidence relating to its effect on the crop yield. Opinion has been freely expressed as to how it acts in the soil, but with little definite agreement. In France especially, investigations have been active on the use of elemental sulphur with a large number of different plants. Work has been done with turnips, beans, celery, lettuce, potatoes, onions, spinach, and other crops. Various results have been obtained, but generally increased yields have been reported. Boullanger and Dugardin (1) place elemental sulphur among the catalytic fertilizers and have reported very favorable results from its use. They are of the opinion that its action is on the soil flora, in some way stimulating the breaking down of organic matter and ammonia production, although their observations show that it has quite a retarding action on nitrification. They further made the interesting observation

that in sterilized soil the addition of elemental sulphur had no effect in increasing plant growth, confirming their idea that elemental sulphur acted through some influence on the soil flora. Demolon (2) believes that sulphur not only acts by stimulating the soil flora but, in addition, acts as a source of needed sulphur after it has been oxidized in the soil. He showed conclusively that flowers of sulphur would gradually oxidize to sulphates in the soil, a statement which we have confirmed and which likewise has been shown by Lint (8) to be true. The fact that elemental sulphur is oxidized in the soil probably has direct bearing on the necessity for the use or presence of adequate quantities of lime or other basic material in a soil receiving this treatment. This may not apply to all crops, but might properly explain the results secured by Wheeler, Hartwell, and Moore (16), who showed that there was injury to cereals following the application of elemental sulphur for the prevention of potato scab, unless a considerable quantity of lime had been used in the soil. From the South Oregon Experiment Station, Reimer (10) reported large increases in the yield of alfalfa by the direct use of elemental sulphur. Whether these experiments were conducted on soils of high basicity has not been reported.

The possibility of injury to the crop by partial oxidation of the elemental sulphur to sulphite must always be kept in mind. Thalau (15) has shown that sulphites of ammonium and calcium are toxic to plants in dilute solution, but probably are not so toxic in the soil itself. The fate of the elemental sulphur introduced into a soil will ultimately be its oxidation to a sulphate, but the formation of intermediate compounds and their toxic effect may account for the contradictory results that have been recorded from its use. For example, Janicaud, Hiltner, and Gronover (7) report deleterious effects with tomatoes from the use of elemental sulphur, and some of the results of Sherbakoff (14) in the treatment of potatoes for scab are of a similar order. Consequently, the attempted introduction of elemental sulphur as a source of sulphur in plant nutrition should, in our judgment, be viewed with caution.

The basis for this statement will be amplified in the following report of experimental work. After this manuscript had been prepared, the work of Shedd (13), of the Kentucky Agricultural Experiment Station, was made public. In this work use was made of a number of sulphates and sulphids, and of elemental sulphur. Good results from the use of a number of these materials are reported. Elemental sulphur and gypsum were helpful to tobacco, and elemental sulphur was materially beneficial to turnips on the soil investigated. Clover on this soil was not helped by sulphur-containing fertilizers, with the exception of a benefit from the use of potassium sulphate. Other plants, such as mustard, cabbage, and radish, showed increased growth with sulphur-containing materials.

EXPERIMENTAL WORK

Beginning in 1911, experiments have been conducted in the greenhouse to determine the influence of sulphates and sulphur on the growth of some common farm crops. Seven different crops representing three different orders have been included in the work up to the present time. They were distributed by orders as follows: Cruciferae—radish (*Raphanus sativus*), rape (*Brassica napus*); Gramineae—oats (*Avena sativa*); barley (*Hordeum vulgare*); Leguminosae—red clover (*Trifolium pratense*), bean (*Phaseolus vulgaris*), pea (*Pisum sativum*). It should be said of plants grown in this way that they sometimes do not develop so well as under field conditions. The lessened light of winter as compared with summer, for example, retards growth, and in the early fall and late spring the day temperatures are likely to become excessive. Also, possibly owing to the protection from wind and the absence of insects, the plants rarely seed well. Despite these influences, however, our crops have grown well in most cases and in some cases have developed luxuriantly. It is true, moreover, that in all cases the effect of varying fertilizer treatments is reliable for comparison, since each crop, save the food supply, was grown under conditions as uniform as possible.

METHOD OF INVESTIGATION

The soil used in this work was the Miami silt loam which predominates on the University Hill Farm. It was obtained by removing the surface vegetation and selecting the surface soil to a depth of about 4 inches. This material was then sifted through a $\frac{1}{4}$ -inch screen and thoroughly mixed. There was practically no loss in the sifting, as hardly a stone was found and the sifted product was smooth and of excellent quality.

A total analysis of the soil showed the following composition, based on the dry matter: Nitrogen (N), 0.15 per cent; phosphorus pentoxid (P_2O_5), 0.14 per cent; sulphur trioxid (SO_3), 0.04 per cent; calcium carbonate ($CaCO_3$), 0.33 per cent; humus, 1.38 per cent.

The humus was determined by the official methods of analysis of the Association of Official Chemists.¹ Fifteen kilos (33 pounds) of this soil were placed in rectangular cypress boxes 16 inches long, 14 inches wide, and 5 inches deep. Seven different fertilizer treatments were tried in duplicate boxes of the soil, as follows:

Boxes Nos.

1-2. Control (no fertilizer).

3-4. Complete fertilizer:

	Gm.
Tricalcium phosphate ($Ca_3(PO_4)_2$)	12.0
Potassium chlorid (KCl)	4.5
Sodium nitrate ($NaNO_3$)	10.0

¹Wiley, H. W., et. al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908.

Boxes Nos.	Gm.
5-6. Complete fertilizer+sodium sulphate (Na_2SO_4)	12
7-8. Complete fertilizer+calcium sulphate (CaSO_4)	12
9-10. Sodium sulphate (Na_2SO_4)	12
11-12. Calcium sulphate (CaSO_4)	12
13-14. Sulphur (flowers)	5

All of these materials were mixed with the soil at the beginning of the experiments, except the sodium nitrate. This was applied in solution in three separate portions as the plants developed. Sulphur was not included in the treatment of the earlier experiments. These amounts of fertilizer are equivalent to the following applications per acre to the surface 8 inches of soil, assumed to weigh 2,000,000 pounds: Tricalcium phosphate, calcium sulphate, and sodium sulphate, 1,600 pounds each; potassium chlorid, 600 pounds; sodium nitrate, 1,330 pounds; and sulphur, 665 pounds.

While these applications may appear excessive as compared with field applications, nevertheless it should be remembered that in these experiments there was a thorough and complete mixing with the entire soil mass. In some cases the soil was limed. For this purpose 10 gm. of calcium carbonate were added to each box in the set. This was at the rate of 1,330 pounds per acre of a depth of 8 inches.

Except in the case of large seeds, such as beans and peas, the seeds were sown liberally in four rows across the boxes and thinned when well developed to 16 plants per box. The larger seeds were germinated on paraffined mosquito netting stretched over distilled water, and transplanted to the soil when well developed. The usual care was taken to support the taller crops and suppress development of fungi and insects, but the use of any sulphur-containing sprays was of course carefully avoided.

When the crops were mature, they were harvested and weighed while fresh. They were then dried quickly in steam-heated trays at about 50° C. and allowed to stand exposed to the air from two to three weeks to become air-dried, in which condition they were finally weighed.

The final comparative weights will be presented in the following tables, in which the weights given are averages obtained from duplicate boxes. In some cases, as indicated, the seed has been separated from the straw and weighed separately. Owing to the difficulty in recovering the roots from the soil, they have been neglected in most cases.

LEGUMINOSAE

BEANS (*Phaseolus vulgaris*).—The variety of beans grown was Davis White Wax. In crop A only 10 plants were grown per box. This crop followed two successive crops of clover on the same soil, the first crop of clover having been fertilized. Crop A was fertilized as usual, except that no sulphur was added to boxes 13 and 14. Crop B was not fertilized. Crop C was completely fertilized. Crop D was grown on a

different set of the same type of soil, but which had produced two crops of rape (both fertilized) and three crops of radishes, the last radish crop having been fertilized. The soils were limed for this crop. The yields of air-dried crops are given in Table I.

TABLE I.—Average weights (in grams) of air-dried bean crops

Treatment.	Seed.					Straw and pods.				
	Crop A.	Crop B.	Crop C.	Crop D.	Average relative yields of crops.	Crop A.	Crop B.	Crop C.	Crop D.	Average relative yields of crops.
1. Control.....	6.4	0.7	7.1	5.3	100	15.7	12.4	34.7	26.8	100
2. Complete fertilizer.....	8.0	5.7	13.8	15.9	223—	21.41	19.1	42.5	45.8	144
3. Complete fertilizer + sodium sulphate.	6.9	3.4	12.9	12.8	185	18.8	20.1	46.8	43.3	144
4. Complete fertilizer + calcium sulphate.	10.4	6.3	17.3	10.1	226—	24.4	22.3	44.3	40.8	147
5. Sodium sulphate only.....	7.1	5.9	13.3	6.6	169	19.2	17.1	31.7	26.0	105
6. Calcium sulphate only.....	6.6	6.1	11.7	4.6	149	14.8	20.2	31.8	21.5	89
7. Sulphur only.....	3.0	4.1	1.9	0.9	51	17.9	20.3	25.3	19.3	92

The relative yields of seed showed irregular results from the application of the sulphates. When added to the usual complete-fertilizer ration, sodium sulphate depressed growth, while calcium sulphate slightly favored it. When applied alone, both salts gave results decidedly better than the control untreated soils. In this case the soluble sodium sulphate gave better results than the comparatively insoluble calcium sulphate. It seems possible that the superior results from the sodium sulphate applied alone as compared with its effect when added to the complete-fertilizer treatment may have been due to an unfavorable excessive accumulation of soluble salts in the latter case which might not occur when it was added alone.

The relative yields of straw from this crop showed no significant effects which might be due to the added sulphates. Sulphur alone was decidedly injurious to the beans. The effect is more noticeable in the case of the grain than with the straw. This might be expected to obtain, since the plants already weakened in general vitality would probably be depressed in the power of reproduction. This was more probably due to sulphites and other toxic oxidation products of the sulphur than to the sulphur itself. It could not be due merely to the acidity of the soil produced by oxidation of the sulphur, for it occurred with crop D, which was limed.

CLOVER (*Trifolium pratense*).—The variety grown was Medium Red. Crop A was grown on fresh fertilized and limed soil. Crop B followed crop A on the same soil without fertilizer treatment, but with the addition of fresh soil in boxes 13 and 14, to which calcium carbonate and elemental sulphur were applied. Crop C was grown on completely

renewed unlimed soil with the usual complete-fertilizer treatment. Crop D was grown on soil which had borne two successive fertilized crops of rape and two successive crops of turnips (*Brassica napus*), the last crop of turnips only receiving fertilizer. This clover crop was limed and fertilized. All the crops were allowed to reach the late-blooming stage, but they failed to produce seed. The roots of crops B and C were separated as carefully as possible from the soil and weighed separately from the tops. The yields of air-dried matter are given in Table II.

TABLE II.—Average weights (in grams) of air-dried clover crops

Treatment.	Hay.					Roots.				
	Crop A.	Crop B.	Crop C.	Crop D.	Average relative yields of all crops.	Crop A.	Crop B.	Crop C.	Crop D.	Average relative yields of crops B and C.
1. Control.....	31.8	56.2	11.7	92.0	100	49.5	16.8	100
2. Complete fertilizer.....	45.3	71.5	48.1	95.0	136	48.5	37.4	130
3. Complete fertilizer+sodium sulphate.....	54.8	72.2	67.0	99.8	153	41.4	31.9	111
4. Complete fertilizer+calcium sulphate.....	46.0	79.2	73.7	108.2	160	48.8	36.4	129
5. Sodium sulphate only.....	33.0	65.9	23.6	93.9	113	67.7	33.1	152
6. Calcium sulphate only.....	27.8	62.5	29.0	116.4	123	92.9	31.9	188
7. Sulphur only.....	49.1	23.6	38	71.9	21.5	141

In the yield of hay there was no doubt about a marked stimulating effect of both sulphates upon growth. Stimulation was equally evident when they were added to the complete-fertilizer treatment and when they were applied alone. In both cases the best results were produced by the less soluble calcium sulphate. Elemental sulphur had a very depressing effect. The average yield from this treatment was but little more than one-third the yield from the control, and in crop D the clover entirely failed to grow where elemental sulphur was applied. Plate XX, figure 1, illustrates the influence of sulphates on the growth of clover.

Root development from the complete-fertilizer treatment was depressed somewhat when sodium sulphate was also applied, but was unaffected when the calcium sulphate was added. We are inclined to ascribe this difference to the depressing effect of the more concentrated soil solution where the soluble sulphate was applied. The effect of the sulphates applied alone was very striking. In Plate XXI is shown the remarkable difference of root development from the different fertilizer treatments. From our limited amount of data calcium sulphate appears to be somewhat more active than sodium sulphate in producing this effect. In any case it appears that in this soil a sulphate has specific effects on the root development of this species. This may properly explain the oftentimes beneficial effects observed in the application of land plaster

to clover. While the form of the root system developed under the two treatments may not involve a larger feeding surface in the one case as compared with the other, yet it does seem very probable that the long root system developed where sulphate concentration was larger would favor that plant in times of limited water supply. The unavoidable conclusion from the results with red clover is that the reenforcement of the limited soil supplies of sulphur compounds by sulphates of sodium and calcium was decidedly beneficial to this crop.

PEAS (*Pisum sativum*).—The variety grown was Little Gem, a dwarf variety. Strong seedlings were transplanted to the soil six days after they were placed on the germinator. The soils had already produced two crops of clover and three of beans, the first crop of clover and the first and last crops of beans having been fertilized. Both clover crops had been limed. No elemental sulphur was added to box 13 and 14 for the first crops of beans. The data of the pea crop are given in Table III.

TABLE III.—Average weights (in grams) of the air-dried pea crop

Treatment.	Seed.	Straw and pods.	Relative yields of seeds.	Relative yields of straw.
1. Control.....	0. 18	4. 42	100	100
2. Complete fertilizer.....	. 21	3. 99	117	90
3. Complete fertilizer+sodium sulphate....	. 24	4. 12	133	93
4. Complete fertilizer+calcium sulphate....	. 97	4. 54	539	103
5. Sodium sulphate only.....	. 60	4. 41	333	100
6. Calcium sulphate only.....	. 82	3. 84	456	87
7. Sulphur only.....	. 03	2. 47	17	56

This crop did not grow vigorously, and the differences of yields have, therefore, less significance than with the preceding crops. However, the increased yields of seeds where sulphates were added is surely remarkable. This is especially true for the calcium sulphate, both when added to the complete fertilizer and when added alone. Both sulphates when applied alone gave remarkable increases over the control soils. Sulphur alone was much more toxic than was the case with the crops already described. The straw shows no very great differences of yields, except where sulphur alone was applied. Here the depressing effect was somewhat less than in the case of the other leguminous crops.

Probably the negative effect of fertilizers upon the growth of straw on this crop should be attributed to the fact that the soils had been excessively cropped and fertilized. This would tend, on the one hand, to exhaust the control soil and, on the other hand, to render the fertilized soils too concentrated in soluble salts for good growth. Hence, the development was even poorer in some cases than the control. Apparently the sulphates especially favored the development of seed in this weakened crop. That such was not the case where sodium sulphate

was added to the complete fertilizer may have been due, as suggested for the previous crops, to a depressing effect of an excess of soluble salts. The favorable effects of calcium sulphate were most decided.

Summarizing the results obtained with the leguminous plants, it may be stated that sulphates added to this soil were decidedly beneficial to the growth of the crops so far investigated. With the large-seeded bean and pea the effects are practically confined to the increased seed development. With the hay crop, however, the results are favorable to the growth of the straw portion of the plant. Calcium sulphate in general is considerably superior to sodium sulphate in its fertilizing action. In the case of clover both of these compounds, when added separately, increased the root development markedly. This would tend to increase the feeding power of the plant and may largely account for the increase of hay produced by their use. Sulphur alone depresses the general development of the plant, with the apparent exception of the clover roots.

CRUCIFERAE

RADISHES (*Raphanus sativus*).—The variety grown was Earliest Scarlet Turnip. Crop A followed two crops of rape on the same soil, both of which had been fertilized. Crop A was not fertilized. Crop B followed crop A on the same soil and was not fertilized. Crop C was also grown on the same soils, but was fertilized. Fifty days from planting crop A, alternate rows of the crop were harvested from one set of boxes for photographing. These were dried and the weights recorded. The remaining plants were allowed to develop seed and the residue rejected. Plate XX, figure 2, is therefore the only available comparison covering the whole crop. The air-dried yields are given in Table IV. (See Pl. XX, fig. 3.)

TABLE IV.—Average weights (in grams) of air-dried radish crops

Treatment.	Crop A.		Crop B (whole plants).	Crop C (whole plants).	Average relative yields of whole plants for all crops.
	Tops.	Roots.			
1. Control	0.2	2.5	19.9	10.3	100
2. Complete fertilizer	1.5	4.7	36.5	34.9	236
3. Complete fertilizer+sodium sulphate ..	1.2	4.7	30.5	48.0	256
4. Complete fertilizer+calcium sulphate ..	1.7	7.0	28.4	47.6	257
5. Sodium sulphate only	1.5	5.0	24.3	10.9	126
6. Calcium sulphate only	1.0	4.7	21.0	11.3	115
7. Sulphur only8	3.7	18.2	7.1	60

The results call for special comment. They show, especially where freshly fertilized (crop C), an unmistakable stimulus to growth by sulphates. The effect is much more pronounced where the sulphates were applied alone than where the complete-fertilizer ration was used. A

point of special interest in these results is the fact that sodium sulphate gave quite as good results as calcium sulphate when added to the complete-fertilizer ration. This suggests that we were dealing here with a plant more tolerant of the concentrated soil solution than were the legumes grown. The radish was also more tolerant of elemental sulphur than were any of the legumes, although the growth in its presence was somewhat inferior to that of the control plants.

RAPE (*Brassica napus*).—The variety grown was Dwarf Essex. Crop A was grown on the usual soil, fresh and completely fertilized except for elemental sulphur. Crop B followed crop A on the same soil. The soil was refertilized and boxes with elemental-sulphur treatment were added. Crop C was grown on fresh-fertilized soil. Crop D followed crop C on the same soil and with the same fertilizer applications. The rape crops were harvested when the death of the basal leaves indicated the near approach of maturity. Data of the weights of the air-dried rape crops are given in Table V.

TABLE V.—Average weight (in grams) of air-dried rape crops

Treatment.	Tops.					Roots.				
	Crop A.	Crop B.	Crop C.	Crop D.	Relative weights with control = 100.	Crop A.	Crop B.	Crop C.	Crop D.	Relative weights with control = 100.
1. Control.....	54.0	12.7	11.6	15.3	100	8.5	2.0	2.7	100
2. Complete fertilizer.....	80.5	29.0	36.4	27.7	188	11.8	3.8	5.1	157
3. Complete fertilizer+sodium sulphate.....	90.0	30.9	45.6	40.9	222	12.3	2.6	5.3	154
4. Complete fertilizer+calcium sulphate.....	78.5	32.9	45.4	50.0	221	12.5	4.9	6.3	181
5. Sodium sulphate alone.....	59.5	13.9	15.8	14.3	111	8.5	2.3	2.8	104
6. Calcium sulphate alone.....	57.0	14.7	13.5	13.3	105	8.8	3.0	3.3	115
7. Sulphur alone.....	13.6	12.3	4.2	32	3.1	2.6	44

It is clearly evident that the addition of sulphates benefited this crop, but especially so where they supplemented the complete-fertilizer ration. Apparently the demands for sulphur of the higher yields of tops from the fertilized plants accentuated the benefits from the sulphates in this case (Pl. XXII, fig. 1).

The sulphates of calcium and of sodium were equally efficient for rape. In the case of the roots only the calcium sulphate gave beneficial results. Possibly the soluble sodium sulphates increased the concentration of the soil solution to such an extent as to retard the growth of the roots. It is well known that in water cultures the roots of plants are more sensitive than the tops to such changes in the nutrient medium. As in water cultures, so, too, in these soil cultures, it appears that the growth of tops and of roots does not proceed parallel.

Rape was also grown upon sand. The sand employed was obtained from the Wausau Quartz Co., Wausau, Wis. It was an angular product, designated as No. 2, which passed almost completely through a sieve of 40 meshes to the inch, but was half retained by a 60-mesh sieve. It contained small amounts of impurities, but no sulphates. Fifteen kgm. (33 pounds) of this sand were placed in the usual boxes with the following fertilizer treatments:

	Boxes Nos.	Gm.
1-2.	Tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$).....	12.0
	Potassium chlorid (KCl).....	4.5
	Magnesium nitrate ($\text{Mg}(\text{NO}_3)_2$).....	2.5
	Sodium nitrate (NaNO_3).....	8.0
	Calcium carbonate (CaCO_3).....	5.0
	Iron chlorid (FeCl_3).....	1.0
3-4.	Like 1 and 2+calcium sulphate (CaSO_4).....	12.0
5-6.	Like 1 and 2+sodium sulphate (Na_2SO_4).....	12.0
7-8.	Like 1 and 2+sodium sulphate (Na_2SO_4).....	6.0

All of the salts, except sodium nitrate, were mixed with the sand before planting, but this was applied to the growing plants in portions from time to time. At 84 days of growth, when the plants gave the usual signs of maturity, the crop was harvested. The yields of the air-dried rape crops are given in Table VI.

TABLE VI.—Average weights (in grams) of air-dried rape crops

Treatment.	Tops.	Roots.	Relative yields when complete fertilizer=100.	
			Tops.	Roots.
1. Complete fertilizer.....	39.0	4.5	100	100
2. Complete fertilizer+calcium sulphate.....	43.0	10.0	110	222
3. Complete fertilizer+sodium sulphate.....	26.5	3.2	68	71
4. Complete fertilizer+ $\frac{1}{2}$ sodium sulphate.....	31.0	3.5	80	78

In these cultures the calcium sulphate was beneficial, but the sodium sulphate depressed the yields as compared with the basal complete fertilization. The data show this effect of the sodium sulphate least where the smaller amount of salt was applied. This again seems to indicate that the depressed effect was due, in part at least, to an excessive concentration of soluble salts. If such an effect were appreciable, one would expect it to be more pronounced in the case of the sand than with soil on account of the lower absorptive power of the former, and such was the case. The calcium sulphate exerted a remarkable effect on the development of the rape roots in these cultures. An objection might possibly be raised that the beneficial effects upon root growth apparent with the soil cultures may have been due to imperfect separation of the

finer parts of the root system from the soil. Such objection would not apply to the sand cultures, which therefore gave conclusive evidence of the stimulating effect of calcium sulphate upon the root development of rape. The benefit to the tops from this salt was much less pronounced, but nevertheless definite. As in most other cases, the elemental sulphur was detrimental to the plants, presumably because of toxic action. There seems to be no doubt that the rape plant has specific need for sulphur, which should be met by including sulphates in its fertilizer treatment.

GRAMINEAE

BARLEY (*Hordeum vulgare*).—One crop was grown upon a set of soils which had already produced one crop of peas with fertilizer treatment and a second crop without fertilizer. The barley crop was not fertilized, as the pea crops had been light. The variety planted was New Zealand Chevalier. In Table VII are given the average air-dried weights of the yields from duplicate boxes.

TABLE XVII.—Average weights (in grams) of air-dried barley crop

Treatment.	Straw.		Grain.	
	Weight.	Relative yields when control=100.	Weight.	Relative yields when control=100.
1. Control.....	36.5	100	9.5	100
2. Complete fertilizer.....	59.0	162	10.5	111
3. Complete fertilizer+sodium sulphate....	67.0	184	14.5	153
4. Complete fertilizer+calcium sulphate....	62.5	171	15.0	158
5. Sodium sulphate only.....	43.5	119	14.0	147
6. Calcium sulphate only.....	38.5	106	17.0	179
7. Sulphur only.....	39.0	107	13.5	142

The limited data available are insufficient for the deduction of definite conclusions concerning the effects of the sulphur supply upon the growth of the barley crop. They indicate, however, that sulphur and the sulphates here applied had little influence upon the production of straw in this crop either when added to a complete-fertilizer ration or when applied alone. Conditions were decidedly different in the case of the grain. While the production of straw seems to have been limited, this amount of straw produced 40 to 80 per cent more grain in the crops receiving sulphur and sulphates alone than in the control crops. Likewise, the crops receiving sulphates in addition to a complete-fertilizer ration produced about 40 per cent more grain than those receiving only the complete ration (Pl. XXII, fig. 2).

OATS (*Avena sativa*).—This crop was grown upon a set of soils which had borne two unsatisfactory barley crops, the first of which had been fertilized. The oat crop was not fertilized. Wisconsin Wonder was

the variety planted. Unlike the barley, this grain crop showed decided differences in development upon the different rations during its growth, as shown in Plate XXII, figure 3. In Table VIII are given the average yields of the thrashed crop in the usual manner, the husks being carefully removed from the seed.

TABLE VIII.—Average weights (in grams) of the air-dried oat crop

Treatment.	Straw.		Grain.	
	Weight.	Relative yields when control=100.	Weight.	Relative yields when control=100.
1. Control.....	28.5	100	2.5	100
2. Complete fertilizer.....	56.0	197	5.0	200
3. Complete fertilizer+sodium sulphate.....	57.5	202	8.5	340
4. Complete fertilizer+calcium sulphate.....	54.5	191	8.5	340
5. Sodium sulphate only.....	19.5	68	2.5	100
6. Calcium sulphate only.....	19.0	67	2.5	100
7. Sulphur only.....	23.5	82	3.5	140

The statements previously applied to the limited amount of data on barley also apply to the oats. So far as the preceding table is concerned, however, it indicates, as in the case of barley, no appreciable effect of sulphates upon the development of straw when they supplement the usual complete-fertilizer ration. Sulphur and sulphates alone even depressed the yield of straw as compared with the control crops.

In the case of the grain, the application of sulphur and sulphates alone did not increase the yield as compared with the controls, although it increased the ratio of grain to straw. The crops receiving complete fertilizer indicate a marked stimulating effect of sulphates upon seed production in this crop. Those crops receiving sulphates in addition to a complete fertilizer produced 70 per cent more seed than those receiving complete fertilizer only.

The data from these two crops of the Gramineae family have shown a marked response of these plants to the application of sulphates by increased seed production. From these records it appears that under present common methods of fertilization these grain crops may frequently reach a maximum production of straw, but that the capacity of this yield of straw to produce seed may be greatly enhanced by the addition of calcium sulphate or sodium sulphate to the so-termed complete-fertilizer ration. In future investigations the writers plan to determine whether the indications here obtained with the Gramineae express a general and fundamental sulphur requirement of this family of plants.

The influence of the concentration of the soil sulphates on the sulphur content of plants has already received consideration (9), but it will not

be out of place to include further data on that subject. Work has been done especially on clover and rape. Data illustrating this influence are given in Table IX. The crops were air-dried.

TABLE IX.—*Influence of supply of sulphates on the sulphur and potassium content of clover and rape*

Treatment.	Clover tops.								Rape.					
	Crop B.			Crop E.					Crop B.			Crop D.		
	Sulphur.	Crop.	Quantity of sulphur removed.	Sulphur.	Crop.	Quantity of sulphur removed.	Potassium.		Sulphur.	Crop.	Quantity of sulphur removed.	Sulphur.	Crop.	Quantity of sulphur removed.
1. Control.....	Pr. ct.	Gm.	Gm.	Pr. ct.	Gm.	Gm.	Pr. ct.	Pr. ct.	Gm.	Gm.	Pr. ct.	Gm.	Gm.	Gm.
2. Complete fertilizer.....	0.15	56	0.084	0.20	28	0.056	1.58	0.60	12	0.072	0.22	15	0.033	0.033
3. Complete fertilizer + sodium sulphate.....	.20	71	.142	.14	85	.119	2.42	.18	29	.054	.22	27	.059	.059
4. Complete fertilizer + calcium sulphate.....	.20	72	.144	.21	99	.207	2.63	.87	31	.269	.78	41	.319	.319
5. Sodium sulphate only.....	.20	79	.558	.25	110	.275	2.31	.90	33	.290	.70	50	.350	.350
6. Calcium sulphate only.....	.11	66	.072	.13	56	.072	1.64	1.18	14	.165	1.08	15	.162	.162
7. Sulphur only.....	.16	63	.100	.25	61	.152	1.36	1.18	14	.165	.90	13	.117	.117
	.19	49	.093	.22	45	.099	1.32	1.00	13	.130	1.66	4	.066	.066

As has been pointed out, the effect of a more concentrated soil-sulphur solution is to increase the total sulphur content of the root and the stem, but not of the seed. This influence is particularly great in the case of the leafy plant like the rape, but is not so marked in the red clover. In the rape the percentage variation of sulphur ranged from 0.20 to 1, depending upon the supply, while in the clover the range was from 0.10 to 0.20. In the case of one crop of clover there is included the total potassium content of this crop. It has been common, since the time of Boussangault, to explain the action of calcium sulphate in the soil as a liberator of potassium, and its effect as indirect. This explanation might still be used for our own results where calcium sulphate was added alone. In this case the growth of crop was so much increased over the growth in the control that the total potassium removed was considerably more than in the control. But where the complete fertilizer containing potassium chlorid is compared with the complete fertilizer plus calcium sulphate, such an explanation for the action of calcium sulphate becomes untenable. The increased growth due to the calcium sulphate in the presence of a complete fertilizer containing potassium can have no other explanation than that its action was direct rather than indirect.

SUMMARY

The data presented from these greenhouse studies with one type of soil indicate that certain plants are measurably increased in their growth by the addition of sulphates. We have emphasized in another

place the fact that sulphates have very little effect as compared with soluble phosphates on the soil flora. This difference in action will remove the sulphates from the category of effective fertilizers for all crops. Nevertheless, for certain plants and types of soil they will be beneficial if their only action is as a source of sulphur.

The plants most affected were the members of the Leguminosae and Cruciferae. It is probable that we should expect these classes of plants to be more responsive to the higher concentration of sulphates in the soil water than, for example, the Gramineae, owing to the higher protein content of the first group and the special sulphur-bearing bodies abundantly formed in the second group. In this soil, however, there was noticeable stimulation to seed production in both barley and oats, although there was little or no effect on the development of the quantities of straw.

In the case of clover the increase in air-dried matter due to calcium sulphate alone was about 23 per cent. With rape the greatest increase occurred where the calcium sulphate was superimposed upon a complete fertilizer, giving an increase of 17 per cent over the complete fertilizer. A similar order of increase was likewise observed with the radish crop, where the increase above a complete fertilization, due to the calcium sulphate addition, averaged 9 per cent.

In general, the calcium sulphate was more effective than the more soluble sodium sulphate. The special influence of sulphates on root development is pointed out. They were particularly effective with red clover and rape. In the case of red clover, which was more especially studied, the roots were much elongated where sulphates entered into the ration. This must result in a more extended feeding area for the plant and, in addition, increase its ability to withstand periods of drought.

The somewhat common observation of the benefit of land plaster to this plant can probably be closely correlated with this special effect of sulphates on root development, as well as its high protein character, which would make special demands for sulphur.

Whether recorded failures in the use of land plaster are to be correlated with wet seasons, a high sulphur content normal to the soil under observation, or the variety of plants used is a matter for future observation.

In these greenhouse experiments elemental sulphur was generally harmful. These harmful results occurred even in the presence of a generous supply of calcium carbonate. These results indicate that elemental sulphur may be toxic through its incomplete oxidation to sulphites; toxicity may also arise in the absence of sufficient basic material through the development of acidity from sulphuric acid.

Application of these results to field practice is reserved until more data on field plots are available.

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PLATE XX

Fig. 1.—Clover plants, showing influence of sulphates on growth. 1, Check; 2, nitrogen, phosphorus, potassium; 3, nitrogen, phosphorus, potassium, plus sulphur as sodium sulphate; 4, nitrogen, phosphorus, potassium, plus sulphur as calcium sulphate; 5, sodium sulphate only; 6, calcium sulphate only; 7, elemental sulphur only.

Fig. 2.—Radish plants, showing influence of sulphates on growth. 1, Check; 2, nitrogen, phosphorus, potassium; 3, nitrogen, phosphorus, potassium, plus sulphur as sodium sulphate; 4, nitrogen, phosphorus, potassium, plus sulphur as calcium sulphate; 5, sodium sulphate only; 6, calcium sulphate only; 7, elemental sulphur only.

Fig. 3.—Radish plants, showing influence of sulphates on growth. 1, Check; 2, nitrogen, phosphorus, potassium; 3, nitrogen, phosphorus, potassium, plus sulphur as sodium sulphate; 4, nitrogen, phosphorus, potassium, plus sulphur as calcium sulphate; 5, sodium sulphate only; 6, calcium sulphate only; 7, elemental sulphur only.



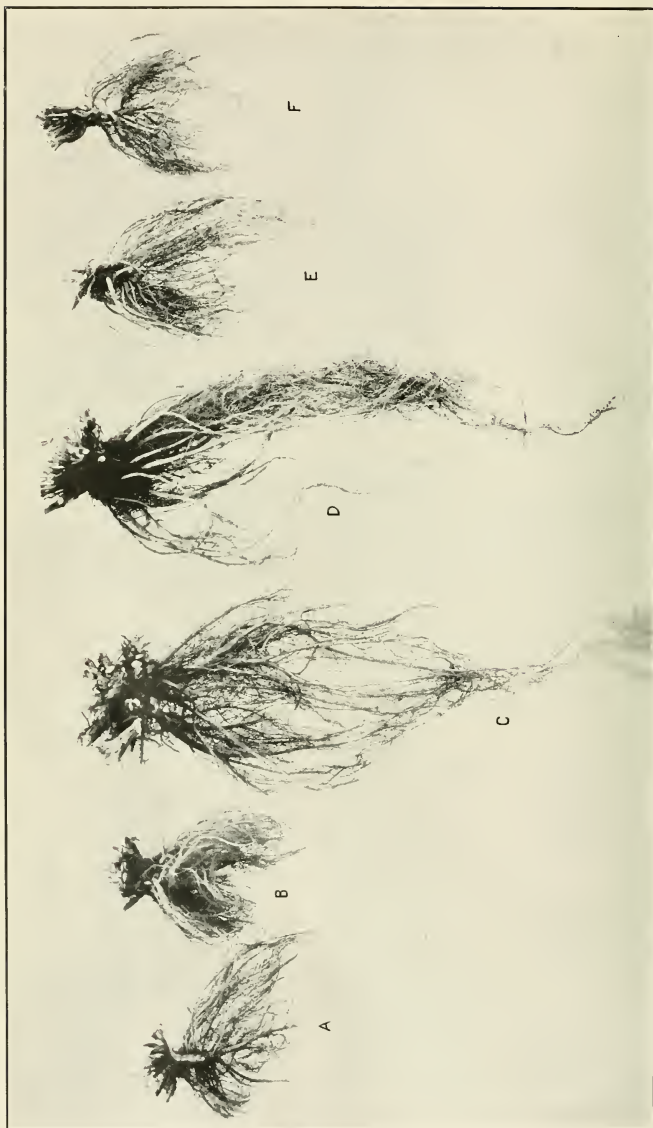


PLATE XXI

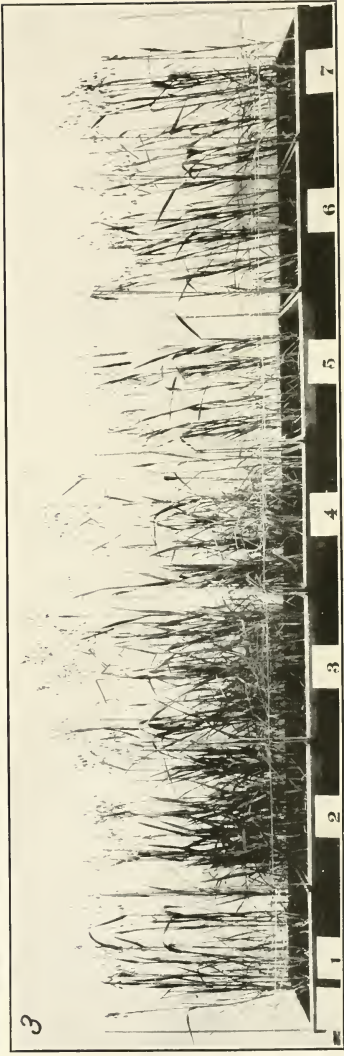
Red clover, showing effect of sulphates on growth of roots. *A*, Check; *B*, nitrogen, phosphorus, potassium; *C*, nitrogen, phosphorus, potassium, plus sulphur as sodium sulphate; *D*, nitrogen, phosphorus, potassium, plus sulphur as calcium sulphate; *E*, sodium sulphate only; *F*, calcium sulphate only.

PLATE XXII

Fig. 1.—Rape plants, showing influence of sulphates on growth. 1, Check; 2, nitrogen, phosphorus, potassium; 3, nitrogen, phosphorus, potassium, plus sulphur as sodium sulphate; 4, nitrogen, phosphorus, potassium, plus sulphur as calcium sulphate; 5, sodium sulphate only; 6, calcium sulphate only; 7, elemental sulphur only.

Fig. 2.—Barley plants, showing influence of sulphates on growth. 1, Check; 2, nitrogen, phosphorus, potassium; 3, nitrogen, phosphorus, potassium, plus sulphur as sodium sulphate; 4, nitrogen, phosphorus, potassium, plus sulphur as calcium sulphate; 5, sodium sulphate only; 6, calcium sulphate only; 7, elemental sulphur only.

Fig. 3.—Oat plants, showing influence of sulphates on growth. 1, Check; 2, nitrogen, phosphorus, potassium; 3, nitrogen, phosphorus, potassium, plus sulphur as sodium sulphate; 4, nitrogen, phosphorus, potassium, plus sulphur as calcium sulphate; 5, sodium sulphate only; 6, calcium sulphate only; 7, elemental sulphur only.



DISTRIBUTION OF THE VIRUS OF THE MOSAIC DISEASE IN CAPSULES, FILAMENTS, ANTHERS, AND PISTILS OF AFFECTED TOBACCO PLANTS

By H. A. ALLARD

*Assistant Physiologist, Tobacco and Plant-Nutrition Investigations,
Bureau of Plant Industry*

Embryonic transmission of the mosaic disease from parent to offspring has not been observed in tobacco plants. Although the disease sometimes appears particularly malignant, so that normal capsule development is almost completely inhibited and few viable seed are produced, plants from such seed are healthy. The normal reproductive vigor of tobacco plants may not be seriously checked by the mosaic disease, especially if it makes its appearance late in the development of the plant. In such plants a nearly normal vegetative development has been attained and subsequent flowering and seed production appear to be little, if at all, inhibited.

It is of considerable interest to know how closely the embryo may be invested with tissues bearing the infectious principle of the mosaic disease. Before the question had been fully investigated the writer was under the impression that the virus ordinarily did not reach the placental column bearing the seeds. In order to test this point, three healthy Connecticut Broadleaf tobacco plants were set aside until seed production had begun. The spongy placental tissue of six to eight capsules on each plant was then punctured deeply with a needle and the virus of mosaic disease introduced abundantly. Capsules of all ages, from very young to those fully grown, were punctured and the virus injected. Although a number of the more immature capsules developed very poorly following this treatment, an abundance of seed was secured and sowed on March 31, 1914. From this seed 400 plants were obtained and later transplanted to 3-inch pots. On May 18 all were healthy, and 40 were inoculated with the virus of the mosaic disease. Practically all of those inoculated were showing symptoms of the disease on May 27 and 28.

Later experiments with affected plants have shown that the capsules of such plants normally contain the virus of the disease. The tobacco capsule contains two cells formed by a median cross wall or partition. By cutting through the thin ovary wall near this partition on both sides of the capsule the ovary wall can be readily removed in two halves, exposing to view each half of the large placental column with its attached

ovules. A thin, sharp scalpel heated to redness was used for cutting away the ovary wall, so that possible infection of any portion of the placental tissues from the ovary wall itself was avoided. Table I shows the occurrence of virus in the placental structure and ovules of mosaic-diseased plants.

TABLE I.—Occurrence of virus in the placental structure and ovules of tobacco plants affected with the mosaic disease

Date of inoculation.	Number of plants.	Variety.	Material used for inoculation.	Effect.
1914. Apr. 23	10.....	Connecticut Broadleaf.	Sap of portions of placental column and immature ovules of green capsules from plants affected with mosaic disease. These portions were macerated in a mortar with clean tap water.	8 affected with mosaic disease on May 9.
23	10.....	do.....	Sap of green leaves from same plants.	6 affected with mosaic disease on May 9.
23	10(control)...	do.....	Sap of green placentas and ovules from a healthy plant and macerated with tap water.	All healthy on May 9.
May 18	10.....	Maryland Mammoth.	Sap of macerated placentas and immature ovules of large, green capsules of plants affected with mosaic disease.	6 affected with mosaic disease on May 26.
18	10.....	do.....	Sap of ovaries entire from the same plants.	10 affected with mosaic disease on May 26-28.
18	10.....	do.....	Thin paste obtained by grinding in a mortar with a small quantity of tap water the white and brownish immature seeds of two capsules from plants affected with mosaic disease. These seeds were scraped very carefully from the placental column.	4 affected with mosaic disease on May 28.
18	10.....	do.....	Sap of two placentas alone, from which the ovules were removed in the preceding test.	7 affected with mosaic disease on May 28.
18	10(control)...	do.....	Sap of immature seeds and placentas obtained from a healthy plant and ground with tap water.	All healthy on May 28.
28	10.....	do.....	Macerated placentas and immature seeds of green capsules from plant A, affected with mosaic disease.	10 affected with mosaic disease on June 6.
28	10.....	do.....	Thoroughly mature, loose seeds from dried, brown, matured capsules of the same plant, A, were poured from the capsules into a mortar and ground to a thin paste with tap water.	3 affected with mosaic disease on June 8.
28	10(control)...	do.....	Macerated placentas and immature seeds of green capsules from a healthy plant, mixed and ground in a mortar with dried mature seeds from the same plant. A small quantity of tap water was added to obtain a thin paste.	All healthy on June 8.
June 2	10.....	do.....	Macerated white immature ovules carefully removed from the spongy, succulent placentas of green capsules of plants affected with mosaic disease and mixed with tap water to form a thin paste.	4 affected with mosaic disease on June 10.
2	10.....	do.....	Sap of leaves from the same plants affected with mosaic disease used in the preceding test.	10 affected with mosaic disease on June 10.

TABLE I.—Occurrence of virus in the placental structure and ovules of tobacco plants affected with the mosaic disease—Continued

Date of inoculation.	Number of plants.	Variety.	Material used for inoculation.	Effect.
1914. June 2	10.....	Maryland Mammoth..	Thin paste obtained by grinding with tap water in a mortar thoroughly dry, loose, ripened seeds from matured capsules of plants affected with mosaic disease.	4 affected with mosaic disease on June 10.
2	10.....	do.....	Same macerated material used as in preceding test.	2 affected with mosaic disease on June 10.
2	10 (control)...	do.....	Paste obtained by grinding together white immature ovules from green capsules and dry, loose, ripe seeds from healthy plants. Small quantity of tap water added to thin the paste.	All healthy on June 10.
June 4	10.....	do.....	Thin paste obtained by grinding with tap water loose, dry, thoroughly ripened seeds of capsules from plants affected with mosaic disease.	7 affected with mosaic disease on June 10.
4	10.....	do.....	Sap of green leaves from the plants in the preceding test.	10 affected with mosaic disease on June 10.
4	10.....	do.....	Paste obtained by grinding and thinning with tap water dry, loose, ripe seeds from capsules of plant B affected with mosaic disease.	7 affected with mosaic disease on June 10.
4	10.....	do.....	Thin paste obtained by grinding with tap water the nearly mature, light brown seeds from ripening capsules of the same plant B affected with mosaic disease. In this test the capsules selected were still green and the placental column succulent and full. The seeds, which were firm and brownish in color, still adhered to the surface of the placenta.	3 affected with mosaic disease on June 10.
4	10 (control)...	do.....	Paste obtained by macerating in a mortar with tap water dry, loose seeds, nearly matured seeds, and leaves of healthy plants.	All healthy on June 10.
5	10.....	do.....	Paste obtained by macerating with tap water the loose, dry seeds from ripening capsules of plants affected with mosaic disease. These seeds were mature, but the placental column was still succulent, although beginning to dry and shrink somewhat.	2 affected with mosaic disease on June 11.
5	10.....	do.....	Macerated placentas from which the seed in the preceding test was removed. Small quantity of tap water added to obtain a thin paste.	8 affected with mosaic disease on June 11 and 12.
5	10 (control)...	do.....	Paste obtained by macerating with tap water in a mortar the dry, loose seeds and placentas of capsules obtained from healthy plants.	All healthy on June 11 and 12.

Earlier experiments¹ have shown that the roots, the apparently healthy lower leaves, and the corollas of plants affected with the mosaic disease sooner or later carry the virus of the disease. More recently experiments have been carried out to determine whether the virus is present

¹ Allard, H. A. Mosaic disease of tobacco. U. S. Dept. Agr. Bul. 40, p. 18-19. 1914.

in the filaments, anthers, and pistils of blossoms produced by affected plants. See Table II.

TABLE II.—*Occurrence of virus in the filaments, anthers, and pistils of blossoms produced by tobacco plants affected with the mosaic disease*

Date of inoculation.	Number of plants.	Variety.	Material used for inoculation.	Effect.
1914. May 21	10.....	Maryland Mammoth..	Sap of macerated pistils extracted very carefully with forceps from the blossoms of a tobacco plant affected with mosaic disease. A gentle pull with the forceps readily severs the style at its junction with the apex of the ovary.	10 affected with mosaic disease on May 28.
21	10.....do.....	Sap of leaves of the same plant, A.	Do.
21	10(control).....do.....	Sap of the leaves and pistils of a healthy plant.	All healthy on May 28.

Experiments with the pistils of plants affected with the mosaic disease were again repeated, using only the upper portion of the style and the stigma. This was done to avoid the possibility of infection from tissues of the ovary adhering to the base of the style when extracted. See Table III.

TABLE III.—*Occurrence of virus in the upper portions of the filaments, anthers, and pistils of blossoms produced by tobacco plants affected with the mosaic disease*

Date of inoculation.	Number of plants.	Variety.	Material used for inoculation.	Effect.
1913. May 27	10.....	Maryland Mammoth..	Macerated upper portions of pistils from plants affected with mosaic disease.	8 affected with mosaic disease on June 6.
27	10.....do.....	Sap of leaves of the same plants.	10 affected with mosaic disease on June 6.
27	10(control).....do.....	Sap of leaves and upper portions of the pistils of healthy plants.	All healthy on June 6.
June 2	10.....do.....	Sap of anthers of plants affected with mosaic disease. These anthers were carefully removed with forceps just prior to opening, and were macerated in a mortar with a small quantity of clean tap water sufficient to make a thin paste.	10 affected with mosaic disease on June 10.
2	10(control).....do.....	Sap of anthers of healthy plants extracted in the same manner.	All healthy on June 10.

From the preceding experimental data it is evident that the virus of the mosaic disease in affected plants becomes distributed throughout the placental structures, reaching even the ovules themselves. Whether the virus passes beyond the integuments of the ovules to the embryo sac has not been determined. There is some indication that the macerated placenta in a succulent condition is more effective than the immature

ovules, and especially the loose, dry, normally ripened seeds, in producing the mosaic disease in inoculated plants. Although the greatest care may be exercised in removing immature seeds from a succulent placental column, it must be evident that the probability of rupturing and removing some of the placental substance is very great. In the normal ripening process, however, the seeds loosen and fall away from the drying and shrinking placental column so gradually that the minimum amount of placental material is carried away attached to the seeds.

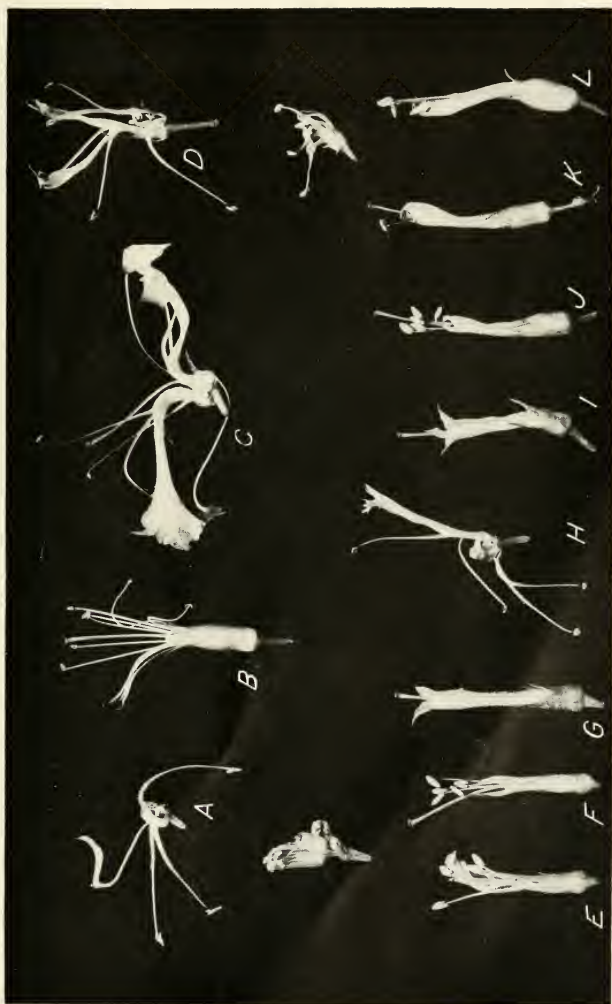
Malformations caused by the mosaic disease may disturb the normal relations of stamens and pistils to such an extent as to cause sterility in many blossoms, owing to the failure of natural self-pollination. Hand pollination of these pistils has frequently led to normal seed development. Not infrequently the development of the corolla is almost entirely inhibited and the stamens and pistils also fail to develop normally. Even in these blossoms the anthers may contain more or less functioning pollen, which has produced normal fertilization when transferred to the pistils of healthy blossoms. In some instances the anthers produce little or no functioning pollen. In extreme cases the normal form and structure of the anther sacs is replaced by a mass of irregular proliferations. Generally blossoms affected with the mosaic disease appear to produce viable pollen and ovules quite as freely as those borne by healthy plants (Pl. XXIII).

From the fact that the mosaic disease is not known to occur as the result of embryonic transmission of the disease directly from the mother plant during seed development, it is evident that a very efficient barrier guards against embryonic infection or the subsequent successful continuation of the disease from parent to seedling. In particularly malignant cases of the disease, where few or no viable seed are produced, following pollination with pollen from healthy blossoms, it is possible that the infective agents of the disease have produced embryonic infection which resulted in death. Whether the failure to produce viable seed in these instances is due to actual infection of the ovules or to a general impairment of nutrition and cell division of the capsular structures associated with embryonic development, can not at present be determined. It is possible that embryonic development never proceeds in those ovules actually invaded and infected by the virus of the disease. In all experimental tests at least germinable seeds from plants affected with the disease have always produced normal, healthy offspring.

At this time speculation seems quite fruitless, and one can only wonder what protects the embryo so securely from the mosaic disease, even though intimately associated with and nourished by infective parental tissues.

PLATE XXIII

Malformed blossoms of tobacco (*Nicotiana tabacum*) caused by the mosaic disease, which is often responsible for the various abnormalities shown. The corolla may show mottling only, or it may develop very imperfectly, producing various degrees of catacorolla, fasciation, etc. In some instances the corolla fails to develop entirely. The plants producing these acquired abnormalities as a result of the mosaic disease have been studied as to their inheritance, but the descendants were healthy and their blossoms normal. A common cause of sterility is the failure of successful pollination of the stigma, owing to the abnormal displacement of pistil and stamens. Hand pollination of such blossoms has often given capsules containing an abundance of fertile seed. Blossoms as poorly developed as A, D, and H are usually incapable of producing seed. The anthers, however, sometimes contain functioning pollen which may produce fertilization of the ovules when transferred to the pistils of healthy blossoms. Blossoms E, F, G, I, J, K, and L usually produce seed if hand pollination is practiced.



DISSEMINATION OF BACTERIAL WILT OF CUCURBITS

[PRELIMINARY NOTE]

By FREDERICK V. RAND,

Assistant Pathologist, Laboratory of Plant Pathology, Bureau of Plant Industry

In the discussion of his exhaustive studies upon bacterial wilt of cucurbits, Dr. Erwin F. Smith¹ makes the following statements relative to certain still unsolved portions of the wilt problem:

Leaf-eating insects, and especially *Diabrotica vittata* (fig. 55), are, I believe, the chief agents in the spread of this disease. They feed readily, and sometimes the writer has thought preferably (fig. 7), on wilted leaves which are swarming with this organism. In this way their mouth-parts can not fail to become contaminated and to serve as carriers of the sticky infection. No other means of dissemination is known to the writer, and this is believed to be the common way in which the disease is distributed. * * *

Seasonally the disease does not manifest itself until the leaf-eating beetles have put in their appearance, and this has led to the suspicion that the organism might pass the winter inside the bodies of these hibernating insects (*Diabrotica vittata*). As to this nothing definite is known.

He has referred to this subject again in his St. Louis address,² as follows:

The writer has since proved several diseases to be transmitted by insects, notably the wilt of cucurbits, and here the transmission is not purely accidental, but there appears to be an adaptation, the striped cucumber beetle (*Diabrotica vittata*), chiefly responsible for the spread of the disease, being fonder of the diseased parts of the plant than of the healthy parts. This acquired taste, for it must be that, works great harm to melons, squashes, and cucumbers. Whether the organism winters over in the beetles, as I suspect, remains to be determined. Certainly the disease appears in bitten places on the leaves very soon after the spring advent of the beetles.

It was especially with a view toward throwing some light on the mode of hibernation of the causal bacteria and of developing some practical method of control that the writer undertook to continue the studies upon this frequently very destructive disease. Since the study was begun in midsummer (July, 1914), the first season's work consisted largely of field observations which covered the territory from eastern Long Island, N. Y., and Maryland to Indiana and Wisconsin. Some of the worst examples of injury from wilt were found in eastern Long Island, and accordingly this locality was selected for the field tests of the following season (1915). While further investigations are under way, it appears

¹ Smith, Erwin F. Bacteria in Relation to Plant Diseases. v. 2, p. 215. Washington, D. C., 1911.

² ———. A conspectus of bacterial diseases of plants. In Ann. Mo. Bot. Gard., v. 2, no. 1/2, p. 390, 1915.

desirable to record at this time the result of the first season's experimentation.

At East Marion, Long Island, N. Y., two fields were selected where during the season of 1914 about 75 per cent of the cucumber vines (*Cucumis sativus*) had been destroyed by bacterial wilt, as determined by the writer. Here was an excellent environment in which to test the question as to hibernation of the bacteria in soil *v.* animal carriers. Fifty large frame cages 4 feet square and $3\frac{1}{2}$ feet high were constructed. The lower 18 inches of the sides were boarded up, while the covers and the upper 2 feet were inclosed in 18-mesh wire mosquito netting. These bottomless cages were set 15 inches into the soil, leaving 3 inches of the boarded portion above the soil line. The juncture between cover and sides was sealed with cotton and liquid tar, and the cracks between the boards of the basal portion were stuffed with cotton to prevent access of insects. Twenty-three of these cages were set in one of the fields and twenty-seven in the other. In each field the soil in four cages was sterilized by live steam at 75 pounds' pressure for one hour, but this made no difference in the final result. This was done in order to kill any wilt bacteria which might have wintered over in the soil. In each field the cages containing sterilized and unsterilized soil were located at intervals across the field and cucumbers were planted in the usual way in the soil between and within the cages on June 5 and 6. A half-dozen plants were grown in each cage and later on thinned to three or four. After planting, the cages were all sealed with lead seals to preclude accidental opening of the covers, and whenever necessary to open the cages for examination they were again sealed in the same manner. By this careful construction and setting of the cages it was thought possible to exclude all of the insects injurious to cucumbers except possibly aphides and flea beetles, some of both of which later on entered some of the cages through the wire netting, but were without effect upon the experiment.

Field No. 1 was separated by at least one-half mile, including a quarter-mile depth of woods, from the nearest cultivated cucurbits. It was, in fact, surrounded on three sides by woods and on the fourth side by Long Island Sound.

Field No. 2 was about one-quarter mile from other cucurbits, but without the intervening woodland.

Plate XXIV, figure 1, shows the cages in place in field No. 2; Plate XXIV, figure 2, shows field No. 1, with a cage in the foreground lifted, the darker part of the base indicating the depth buried.

No commercial cucumber fields were planted in either locality until two or more weeks later in the season.

As soon as the young plants were 2 or 3 inches high and before any wilt had appeared, five or six striped cucumber beetles were introduced into each of 4 cages, 2 in each experimental field. These beetles were

collected in field No. 1, where presumably they had hibernated. Within a week several plants in 1 of the 2 cages in field No. 1 into which the beetles had been introduced showed signs of wilt, starting from points in the leaves gnawed by the beetles. Upon cutting off the stems the typical stringing out of the viscid white bacterial slime was seen. Cultures were made by the writer from one of these plants and subsequent inoculations from these cultures into healthy plants again gave the disease. Other wilted plants from the same cage were sent to Washington, D. C., and from one of these *Bacillus tracheiphilus* was obtained and with it successful inoculations were made in cucumbers in one of the Department greenhouses by Dr. Smith. No signs of wilt occurred in the 3 other cages in which beetles were placed, or, with one exception, in any of the 46 other cages.

Meanwhile in both fields the wilt was beginning on plants between the cages. At first the wilt appeared only on a plant here and there, and then gradually extended throughout the two fields until no portion was entirely exempt. In the two fields together there were in the neighborhood of 1,200 hills of cucumbers exposed to attack of the beetles. The cages in field No. 1 extended approximately a quarter of a mile through the field at equal distances and in field No. 2, which was about two-thirds as large, they were spaced closer. There was a check plot contiguous to each cage. The approximate number of cases on the plants in field No. 1 during the three months was 600; in field No. 2 it was 200. No counts were made after September 1, owing to the appearance of the cucumber mildew (*Plasmopora cubensis*).

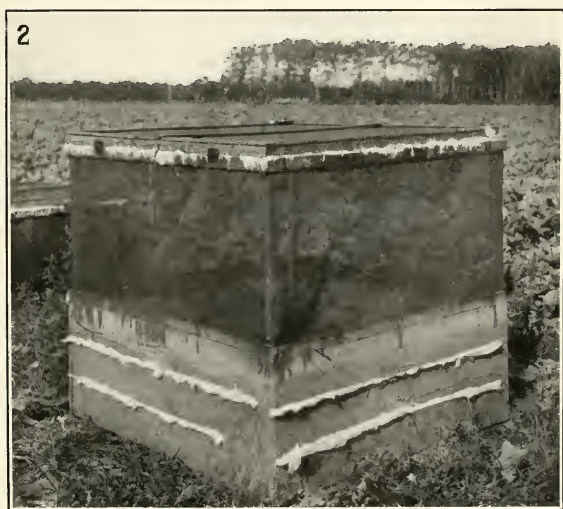
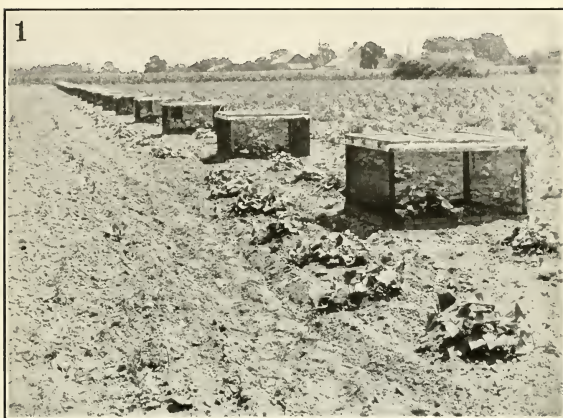
In all these cases of wilt in the exposed (uncovered) plants, infection was clearly seen to have started from beetle injury. Careful record was kept throughout the season of every hill and plant showing wilt, and although between the cages the disease was everywhere present the plants within the cages were strikingly free from the disease. The plants in these 50 cages were examined every day from planting time (June 5-6) until September 1. In one cage where beetles were not liberated, wilt was noted just starting in the tip leaf of one plant at a point gnawed by a beetle. A careful search in this cage disclosed a striped beetle, which was summarily disposed of. Microscopical examination and cultures from the lower part of the stem failed to disclose any bacteria, showing that the wilt in this case could not have come from the soil and must have been brought in by the beetle, which probably entered through a crack due to warping of the boards. Careful search failed to disclose any further beetle injury within the cage, and, after the removal of the beetle and the one wilting plant, no further signs of the disease appeared therein during the season. With this exception and that of the above-mentioned 1 cage into which the beetles were purposely introduced, not a case of wilt occurred in any of the 50 cages during the entire season.

From these cage experiments therefore it would appear that the wilt bacteria are carried over the winter by the hibernating beetles and inoculated into the cucumbers as they feed upon the young leaves. However, from the fact that wilt appeared in only one of the four cages into which beetles were introduced, it would seem that not all hibernating beetles carry the disease, but only those, or some of those, which have previously fed upon wilted plants. In other words, the beetles act not only as summer but also as winter carriers of the wilt organism from one cucumber plant to another. At least the above facts seem to warrant this as a tentative conclusion. The only possible alternative is to suppose that some of the beetles captured on June 17 and introduced into the four cages had recently had opportunity to gnaw diseased plants, which under the circumstances of their capture appears to the writer out of the question. Finally, in addition to the positive evidence of insect transmission afforded by this cage and by the one into which a beetle accidentally penetrated, as well as by daily observation on the check plants, there is the negative evidence afforded by the fact that in all cages from which beetles were excluded the plants remained free from the disease in two fields where it was very prevalent.

PLATE XXIV

Fig. 1.—Cucumber field No. 2, with beetle-proof cages in place.

Fig. 2.—Field No. 1, with one of the cages lifted to show structure of the buried part.



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All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

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GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED MEAL.¹

By W. A. WITHERS and F. E. CARRUTH,²

North Carolina Agricultural Experiment Station

TOXICITY OF COTTONSEED

The term "cottonseed meal" is applied to the ground cake left after the oil is pressed from the seed of cotton (*Gossypium* spp.). For many years it was regarded as a by-product of little value. It is now used extensively as a feed. The annual production of the United States is about 2,000,000 tons, valued at about \$53,000,000. While it may be fed profitably to horses, cattle, sheep, etc., in moderate amounts, poisoning and often death occur as a result, especially if the animal has not been gradually accustomed to it. It is generally avoided as a feed for pigs on account of the numerous deaths associated with its use. Dinwiddie (1905) states that hogs show no greater susceptibility than cattle when fed quantities proportional to their body weight. Feeding experiments at the North Carolina Experiment Station have shown that where swine are fed one part of cottonseed meal with three parts of corn meal death generally ensues in from five to seven weeks, although some pigs have been fed for a year or more without fatal results.

In a recent experiment at this Station nine pigs weighing from 75 to 150 pounds were fed in a closed pen on a daily ration of 1 per cent of cottonseed meal and 3 per cent of corn meal, based on their initial body weight. Six died between the thirty-fifth and the fifty-seventh day. The others were alive on the ninetieth day. Roughly, then, 45 per cent of their initial weight in cottonseed meal was fatal to these pigs. All the smaller pigs died.

Withers and Brewster (1913) found that rabbits and guinea pigs would succumb in about 13 days (6 to 22 days) when fed at the rate of 1 per cent of initial body weight daily. Experiments with 22 rabbits showed that, on

¹ This paper is the third in a series of "Studies in Cottonseed Meal Toxicity." Study I, Withers and Ray (1913), is a criticism of Crawford's pyrophosphoric-acid hypothesis; Study II, Withers and Brewster (1913), suggests iron salts as an antidote.

² For their cooperation with us in this investigation, we desire to thank Dr. G. A. Roberts and Dr. W. B. Smith, of the Veterinary Department, and Dr. B. F. Kaupp, Pathologist, of the Poultry Department, North Carolina Experiment Station.

an average, 8.3 per cent of initial body weight was sufficient to cause death. These authors make the following statement in regard to these tests:

As a rule the rabbits ate the meal well during the first few days and made gains in weight. But towards the end they began to refuse the meal in whole or in part and soon thereafter died.

There have been numerous suggestions as to the cause of poisoning and death from the feeding of cottonseed meal. These are summarized in the Experiment Station Record (1910, p. 501) as follows:

It has been variously ascribed to the lint, the oil, the high protein content, to a toxalbumin or toxic alkaloid, to cholin and betain, to resin present in the meal, and to decomposition products.

Pathogenic organisms and certain fungi have also been suggested.

Friemann (1909), a veterinarian, obtained from the alcoholic extract of cottonseed meal which had caused sickness in cattle a base the platinum salt of which contained 28.75 per cent of platinum. The free base had a paralytic action on exposed frogs' hearts similar to muscarin. He concluded that the toxicity was to be referred to ptomaines which result from the nitrogen-containing components of the lecithin, and that unsaturated fatty acids probably contributed to the total action of the meal.

Crawford (1910) concluded that "the chief poisonous principle in certain cottonseed meals is a salt of pyrophosphoric acid." This conclusion is discussed later in this article.

Withers and Ray (1913b) found that the toxicity of cottonseed meal could be destroyed by boiling it with alcoholic caustic soda. This was the only solvent of a large number used which removed or appreciably affected the toxic principle. A noteworthy fact is that the neutralized and evaporated extract was shown to be nontoxic.

Withers and Brewster (1913) found that if a solution of iron and ammonium citrate was fed with cottonseed meal rabbits did not die during a period about seven times as long as the feeding period when iron salts were omitted. Furthermore, rabbits made sick on the meal recovered when the iron solution was supplied with the meal.

PREPARATION OF GOSSYPOL

Our recent experiments have led us to believe that gossypol is the toxic substance of cottonseed.

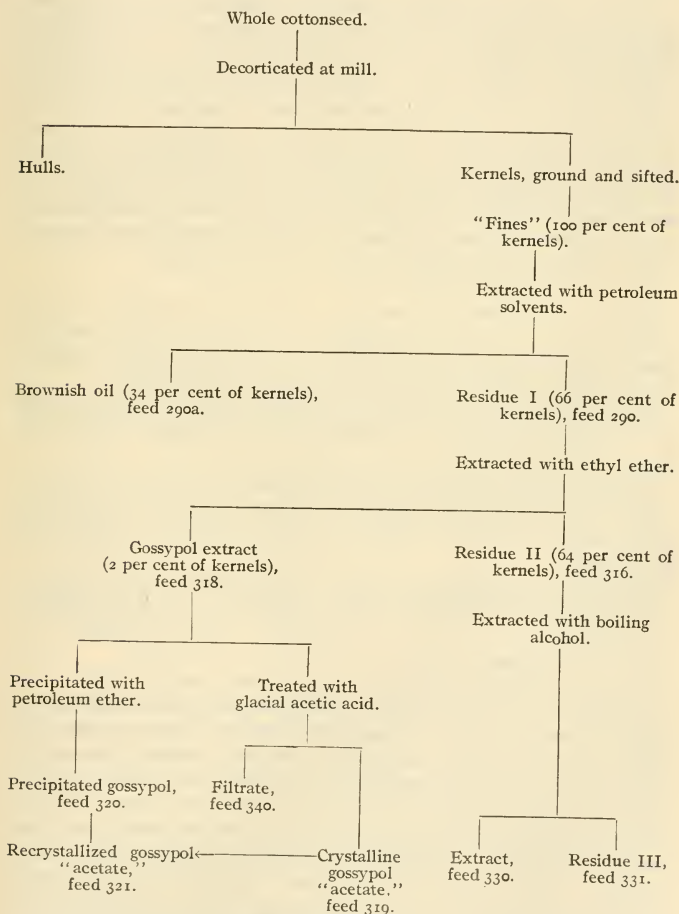
In our previous experiments we used cottonseed meal as the material for study, but in the experiments discussed in this paper we used cottonseed kernels as the initial substance, as gossypol is more readily and more completely extracted from the kernels than from the meal. Generally speaking, the meal and the kernels are toxic to rabbits to the same degree.

We extracted gossypol from ground cottonseed kernels with ethyl ether, after previously removing most of the oil with petroleum ether or gasoline. Gossypol was separated from the ethereal solution by evaporation, by precipitation with petroleum ether, or by precipitation with acetic acid.

These products, differing in purity, have been designated by us as "gossypol extract," "precipitated gossypol," and "gossypol 'acetate.'" All proved toxic to rabbits.

The method of preparing the gossypol and other feeds is shown in the accompanying outline.

OUTLINE OF METHOD OF PREPARING FEEDS



Feeds 290, 318, 319, 320, and 321 are very toxic.
Feed 316 is very slightly toxic after long feeding.
Feeds 331 and 340 are nontoxic.

OCCURRENCE AND PROPERTIES OF GOSSYPOL

If the cottonseed kernel is examined with a lens, many small yellowish brown to black spots may be seen (Pl. XXV). They are referred to by Hanausek (1907, p. 367) as "secretion cavities" in the following statement:

Distributed among the mesophyll cells [of the cotyledons] are procambium bundles and globular, lysigenous secretion cavities (*se*) 100—400 μ diameter. The lysigenous character of these cavities when mature is quite clearly evident. The tissue which surrounds them consists, in its outer portion, of tangentially flattened, very thin-walled cells, and within the last a mucilaginized layer in which the traces of the cell walls are still evident. This colorless mucilage layer, which treatment with hydrochloric acid and, after washing with water, with potash brings out as a yellow, folded, and laminated mass, encloses the greenish-black, opaque secretion (*v*). Since the mucilage layer is soluble in water, the secretion flows out from the sections laid in water in the form of a thick emulsion consisting of a colorless mass containing minute dark-colored grains (resin?) in lively molecular motion. Chlorzinc iodine colors the secretion red-brown, sulphuric acid dissolves it to a thick turbid fluid of a blood-red color. Ammonia colors the liquid greenish yellow without destroying the emulsion. Potash also imparts a green color.

They are designated by Watt (1907, p. 56) as "gland dots" and by Balls (1912, p. 13) as "resin glands." From these glands we have extracted gossypol and for clearness have alluded to them as gossypol glands. Their function does not seem to be very well known.

They occur in all parts of the cotton plant and in all varieties which we have seen. They are very abundant in the cambium layer of the bark of the cotton root.¹

Gossypol was first isolated by Marchlewski (1899) from the "foots" in the purification of cottonseed oil, and on account of its source and phenolic properties he proposed for it the name "gossypol," from Gossyp [ium phen]ol.

Previous to Marchlewski's work the crude substance constituting the coloring matter of cottonseed oil was referred to by the older writers—e. g., Hanausek (1903, p. 755)—as "gossypin,"² which is described as a light-brown pungent powder.

Marchlewski (1899) proposed for gossypol the formula $C_{13}H_{14}O_4$, with $C_{32}H_{34}O_{10}$ as an alternate formula. Among its properties as described by him are the following: A beautifully crystalline yellow-colored dihydroxy phenolic substance, easily soluble in alcohol, benzene, chloroform, ether, acetone, and glacial acetic acid; insoluble in water; soluble in concentrated sulphuric acid with a magnificent red color; easily soluble in alkalis, the solution for the first second being yellow, after a short time becoming a beautiful violet and then fading, the changes being due to oxidation. The alcoholic solution gives a dark-green color with ferric

¹ Thus, we have an indication that gossypol may be the active principle of the medicinal extract of cotton-root bark. (Bouchelle, 1840.)

² The original work on gossypin has not been located by us.

chlorid. The samples dried at 125° to 130°, melted at 179° to 180°, and air-dried samples melted with quick heating at 188°.

Our experiments indicate that the substance which Marchlewski named "gossypol" contained acetic acid in combination with the substance to which we think the name "gossypol" should be assigned. The acetic-acid content of our different products varied from 8.5 to 9.5 per cent, depending upon the conditions under which crystallization took place. The substance containing acetic acid and the substance freed of acetic acid differ in elementary composition and in melting point, as one would expect. Marchlewski's empirical formulæ for gossypol appear to us to be erroneous, as they were based upon the ultimate analysis of the acetate instead of the substance freed from acetic acid.

Marchlewski supposed that gossypol might prove of value as a dyestuff, and before the publication of his article took out patents¹ to protect his discoveries. He made no suggestion as to its physiological activity, nor have we been able to find that anyone else has done so.

EXPERIMENTAL WORK WITH GOSSYPOL

METHOD OF ROUTINE FEEDING

Rabbits and guinea pigs were used in our experiments. Rabbits do not eat cottonseed meal nor cottonseed kernels readily. Therefore, to make the various solid feeds palatable, we moistened them with the best grade of molasses, rabbits eating the various feeds with great relish until made sick. They were fed liberally with green feed once a day.

In case of forced feeding a catheter was inserted to the stomach and the dose allowed to drain in. The intraperitoneal injections were made by the Station veterinarian, Dr. G. A. Roberts, by whom also the post-mortem examinations and notes were made.

The rabbits were fed in galvanized-iron cages, about 20 inches long by 16 inches wide by 10 inches deep. Each contained a trough with separate compartments for water and feed.

TOXICITY OF COTTONSEED KERNELS (FEED 290)

Cottonseed kernels were extracted with petroleum ether, which does not remove gossypol in appreciable quantities. A rabbit was started on 15 gm. daily of this feed, but it would not eat all of it. Diarrhea resulted on the second day, and its appetite for green feed was affected on the third and fourth days. It gradually ate less and less, so that the feed was discontinued on the eleventh day and the ether-extracted kernels (feed 316) substituted on the day following. During the last five days it ate only 11.5 gm. It ate 56.5 gm. of feed 290, losing 130 gm. in weight, but recovered on feed 316.

¹ English patent No. 24418 of 1895 and German patents Nos. 98074 and 98587 of 1898.

Two guinea pigs, A and B, were tried with this feed. Guinea pig A was off its feed at the time from eating precipitated gossypol spread on corn meal (feed 318). An attempt was made to give it kernels in which the gossypol was not so easily detected, but the animal would not touch them.

Guinea pig B had eaten feed 316 for 50 days and had gained in weight. After it had been on corn meal and molasses (feed 317) for about a week, it was placed upon feed 290 (7 gm. of kernels with molasses). It ate only 4 gm. of the kernels, although other feed was withheld for a day. We concluded from this that even the 4 gm. had affected it physiologically and had made it suspicious of the feed. After a week upon control feed, it ate feed 316 without objection.

Rabbit 957, which had eaten feed 316 for 46 days without noticeable effect, was rested for three weeks and then fed the residue after petroleum-ether extraction, which does not remove the gossypol. Its appetite was perceptibly affected on the third day, but it ate most of the feed for 6 days. On the ninth day it refused to eat feed 290, but ate green feed slowly. It died on the fourteenth day, showing symptoms of cottonseed-meal poisoning. See Table I.

TABLE I.—Results of feeding cottonseed kernels (fat-free; feed 290) and cottonseed meal to rabbits and guinea pigs

Feed and animal No.	Weight of animal.			Weight of feed eaten.		Number of days fed.	Result.
	Initial.	Final.	Loss.	Actual.	As kernels.		
Cottonseed kernels:	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>		
Rabbit 958.....	1,560	1,430	130	56.5	85	11	Made sick and refused to eat.
Guinea pig A....	680	0	0	1	Refused feed entirely.
Guinea pig B....	650	4	6	1	Refused the feed.
Rabbit 957.....	1,800	1,535	235	100	150	14	Died.
Cottonseed meal: ^a							
Average for 22 rabbits.	1,577	1,238	339	^b 133	13	All died.

^a The results of Withers and Brewster's experiments (1913) with cottonseed meal are here inserted for comparison.

^b Each rabbit consumed from 48 to 225 gm. of cottonseed meal and died upon the feed in from 6 to 22 days.

TOXICITY OF GOSSYPOL EXTRACT

It is much simpler to prepare gossypol from cottonseed than from the oil.¹ Qualitative tests of ground cottonseed showed that gossypol could be extracted with ether, carbon bisulphid, chloroform, benzene, alcohol, but not with petroleum ether or gasoline. By extracting the

¹ This point will be discussed under the chemistry of gossypol, which will appear in a subsequent publication.

ground kernels in a Soxhlet apparatus for several hours with petroleum ether and then with ethyl ether we obtained a product which for convenience we called "gossypol extract." After the evaporation of the ether there was left a red resinous material which had a peculiar pungent odor and which amounted to about 2.5 per cent of the weight of the kernels used. This material seems to consist largely of gossypol, although we have not yet made an examination with reference to identifying other constituents. No doubt considerable oil is present.

Gossypol extract administered intraperitoneally and fed in one large dose in oil or in small daily doses with corn meal and molasses was found to be toxic to all the animals experimented with.

CATHETER FEEDING OF GOSSYPOL EXTRACT

This gossypol extract from 90 to 180 gm. of cottonseed kernels was fed to each of four rabbits and proved fatal in every case. Care was taken to remove all the solvent, and the gossypol extract was dissolved in cottonseed oil which had been purified in this laboratory. The oil solution was then fed through a catheter. The control animal, on a large dose of cottonseed oil, had diarrhea the next day, but was normal thereafter. Table II summarizes the results obtained with the gossypol extract fed forcibly to rabbits.

TABLE II.—*Results of feeding gossypol extract and purified cottonseed oil with a catheter to rabbits*

Feed and rabbit No.	Weight of rabbit.	Weight of kernels before extraction.	Dose.	Result.
Gossypol extract:	Gm.	Gm.	C. c.	
923.....	1,500	90	15	Died in about 12 hours.
924.....	1,750	180	30 ($\frac{1}{2}$ water.)	Died in 30 to 40 hours.
926.....	3,000	About 160	30-35	Died in 25 hours.
927.....	2,100	170	30 ($\frac{2}{3}$ water.)	Died in less than 16 hours.
Purified cottonseed oil:				
925 (control).....	2,500	30-35	Sick with diarrhea next day only.

POST-MORTEM OBSERVATIONS

Rabbit 923.—Part of dose still in stomach. First foot of intestines considerably injected. Excess serous fluid in abdomen, 10 c. c. No evidence of catheter reaching lungs.

Rabbit 924.—Lungs very much congested. Excess fluid in chest cavity, 3 to 4 c. c. Some hemorrhagic condition along blood vessels of large intestines.

Rabbit 926.—Lungs normal. Anus discolored from diarrhea.

Rabbit 927.—Lungs markedly congested.

INTRAPERITONEAL INJECTION OF GOSSYPOL EXTRACT

Cottonseed oil was used as the vehicle for the injection of the gossypol extract. This was readily available and of suitable consistency for injection. It was purified in this laboratory from a sample of crude oil. This oil was selected chiefly for its ability to hold the gossypol extract in solution. Crawford (1910, p. 531-532), under "Experiments with cottonseed oil," makes the following observations:

After feeding a large dose of the crude cottonseed oil (25 c. c.) to a rabbit its weight steadily fell and remained low, and when a moderate dose (15 c. c.) was fed and this was followed by repeated small ones the animal died, showing irritation of the gastro-intestinal canal. Lendrich [1908] noted that after the daily administration of cottonseed oil his rabbits emaciated, but readily assimilated the same dose of oil that was given intraperitoneally.

After feedings with purified cottonseed oil, or with olive oil, there was a loss in weight, but the animals did not die. After feeding pure cod-liver oil the animals died. The loss in weight was small in the case of feeding purified cottonseed oil. The fact that the cottonseed oil gave no red reaction to litmus paper would suggest that the loss in weight, noted after feeding the crude oil, was not due to the free oleic acid. This acid has recently been shown to play an important rôle in the production of certain forms of anemia. Oils interfere with gastric digestion in man, and this fact must be taken into consideration in experiments on such animals as rabbits.

Two controls receiving purified cottonseed oil were affected to only a slight extent. All five rabbits receiving intraperitoneally an oil solution of gossypol extract died, the extract being the equivalent of from 45 to 85 gm. of cottonseed kernels. See Table III.

TABLE III.—Results of intraperitoneal injection in rabbits of gossypol extract dissolved in purified cottonseed oil

Feed and rabbit [No.	Weight of rabbit.	Weight of kernels before extraction.	Dose.	Result.
Gossypol extract:	Gm.	Gm.	C. c.	
931.....	770	About 50	8	Died.
932.....	600	About 45	15 (½ water.)	Do.
934.....	937	85	5-6	Do.
928.....	1,090	85	7	Do.
929.....	1,225	About 50	4	Do.
Purified cottonseed oil:				
930 (control).....	864	10	Only slightly indisposed.
933 (control).....	864	10	Do.

POST-MORTEM OBSERVATIONS

Rabbits 931 and 932.—Fatal with complications in four days. Entire belly (subcutaneous) very edematous. Part of dose was injected subcutaneously.

Rabbit 934.—Died between the seventh and the nineteenth hour. Considerable serous fluid in abdomen. Serous fluid in chest cavity, 2 to 3 c. c.

Rabbit 928.—Fatal in three hours. Excess discolored serous fluid in abdomen containing oily globules. Moderate injection in intestines at points. Slight excess of fluid. Lungs slightly congested and slightly edematous.

Rabbit 929.—Died during night between the second and the thirteenth hour. Excess brownish serum in abdominal cavity. Small intestines show areas of marked injection. Lungs congested and somewhat edematous.

Rabbit 930.—Slightly indisposed on following day and normal thereafter. Appetite only slightly affected.

Rabbit 933.—Same as 930.

FEEDING GOSSYPOL EXTRACT WITH CORN MEAL AND MOLASSES

An artificial cottonseed meal was made by pouring the concentrated ether extract of cottonseed kernels over corn meal. The daily feed for each of four rabbits was estimated to be equivalent to 30 gm. of cottonseed kernels, and for each of two others, 15 gm. Control animals were given corn meal and molasses. All the animals were supplied liberally with green feed (pea vines, cabbage, and collards) in the morning. In the afternoon (4 or 5 p. m.) they were given the various feeds mixed with molasses. The controls on corn meal and molasses did well, gained in weight, and need not be further mentioned. The gossypol extract proved very toxic. The animals receiving the equivalent of 30 gm. of cottonseed kernels refused to eat the cottonseed feed after the fifth day. They began to refuse green feed later, became sicker, and the last one died within 15 days. The two rabbits and a guinea pig receiving smaller doses were soon made sick. One rabbit and a guinea pig refused the feed thereafter, and the other rabbit died. See Table IV.

TABLE IV.—*Results of feeding gossypol extract (feed 318) with corn meal and molasses to rabbits and guinea pig^a*

DAILY FEED EQUIVALENT TO 30 GM. OF COTTONSEED KERNELS

Feed and animal No.	Weight of animal.			Weight of mixture eaten.		Number of days fed.	Result.
	Initial.	Final.	Loss.	Actual.	As kernels.		
Gossypol extract with corn meal and molasses:							
Rabbit 941...	Gm. 1, 535	Gm. 1, 255	Gm. 280	Gm. 52	Gm. 104	8	Died.
Rabbit 942...	1, 605	1, 250	355	54	108	12	Do.
Rabbit 943...	1, 530	1, 180	350	70	140	11. 5	Do.
Rabbit 944...	2, 095	1, 595	500	71	142	15	Do.
Average...	1, 691	1, 320	371	62	124	11. 7	Do.

^a 1 gm. of the mixture of feed 318 and dry corn meal is equivalent to approximately 2 gm of cottonseed kernels.

TABLE IV.—*Result of feeding gossypol extract (feed 318) with corn meal and molasses to rabbits and guinea pig—Continued*

DAILY FEED EQUIVALENT TO 15 GM. OF COTTONSEED KERNELS

Feed and animal No.	Weight of animal.			Weight of mixture eaten.		Number of days fed.	Result.
	Initial.	Final.	Loss.	Actual.	As kernels.		
Gossypol extract with corn meal and molasses:							
Rabbit 953...	Gm. 1,915	Gm. 1,755	Gm. 160	Gm. 41	Gm. 82	11	Died. Experiment discontinued.
Rabbit 954...	1,790	1,740	50	80	160	20	
Gossypol extract alone:							
Guinea pig A	770	650	120	34	68	29	Do.

POST-MORTEM OBSERVATIONS

Rabbit 941.—Reddish serum in abdominal cavity, 15 c. c. Cecum deeply injected. Liver congested. Lungs slightly congested and edematous. Conspicuous thrombus in right auricle.

Rabbit 942.—Excess abdominal fluid, 15 c. c. Hemorrhagic (inflamed) and ulcerated condition at pyloric end of small intestines. Large thrombus in right auricle.

Rabbit 943.—Slight excess of abdominal fluid. Large intestines had some hemorrhagic areas. Liver congested.

Rabbit 944.—Reddish serum in abdomen, 25 c. c. Serous membrane injected. Small intestines reddened. Small thrombi present. Death due to enteritis.

Rabbit 953.—Mesenteric blood vessels injected. Viscera practically normal. Liver much congested. Kidneys much congested.

Rabbit 954.—Experiment discontinued because animal refused to eat feed 318 after the eighth day. Subsequently put on precipitated gossypol.

Guinea pig A.—Experiment discontinued because animal refused to eat feed 318.

In order to ascertain the effect of a large dose, a large healthy rabbit (945) was taken from the control feed and given all of feed 318 that it would eat. It consumed 40 gm., equivalent to 80 gm. of kernels, on the first day and was made sick on the following day. When it began to recover on the fourth day it was given a small feed and died on the ninth day, having lost considerably in weight. The protocol of rabbit 945 is as follows:

September 23, p. m.—Ate 40 gm. of feed 318 with molasses.

September 24.—Appears sick; has diarrhea. Ate little green; refuses feed 318.

September 25.—Seems indisposed; refuses feed 318.

September 26.—Better; eats cabbage. Weight 2,700 gm. Given 15 gm. of feed 318 and 15 gm. of corn meal with molasses. Ate equivalent to 7 gm. of feed 318.

September 27, 28, and 29.—Eats pea vines readily.

September 30.—Refuses green; p. m., ate corn meal and molasses readily.

October 1, a. m.—Refuses green. Died ninth day about 3 p. m. Weight, 2,410 gm.

Post-mortem examination showed considerable excess fluid in abdominal cavity.

TOXICITY OF PRECIPITATED GOSSYPOL

By the term "precipitated gossypol" we designate a product obtained from the gossypol extract. In securing the extract in larger quantities the oil was not entirely removed from the cottonseed kernels by several previous extractions with gasoline; hence, the gossypol extract contained considerable amounts of oil. The dark-red oily gossypol extract, after evaporation of the ethyl ether, was mixed with a large quantity of petroleum ether. Under some conditions a part of the gossypol precipitated in brown flocks, which could be separated easily by filtration. Under conditions of rapid precipitation these flocks would agglomerate and form a red resinous material. Both the light-brown powder and the red resinous material dissolved in ether very readily, giving a deep cherry-red solution.

Another artificial cottonseed meal was prepared by dissolving weighed quantities of precipitated gossypol in ether, pouring the solution over corn meal, and warming over a steam bath to drive off the ether. One gm. of precipitated gossypol was usually mixed with 50 gm. of corn meal. This proportion was based on the assumption that gossypol existed in cottonseed kernels to the extent of 2 per cent.

Our earlier estimate of 2 per cent appears to be too high. The largest yields of crystalline gossypol acetate secured from the extract were from 0.8 per cent to 1 per cent of the weight of the kernels. This probably represents nearly the entire amount present, as very little gossypol is dissolved by gasoline and little is left after ether extraction, judging by the slight toxicity of the residue.

Pouring the deep cherry-red solution over corn meal gave it a red color. When this was warmed over the steam bath, the color of the corn meal changed to a typical cottonseed-meal yellowish brown. No explanation is offered for this change; but it is evidently not due to oxidation, as the change begins at the bottom of the mixture, not at the surface.

This artificial meal was fed to six rabbits and proved fatal in every case. We had difficulty in getting them to eat it after having been once made sick.

Rabbit 954 was taken from feed 318 (gossypol extract) and offered corn meal and molasses containing 0.37 gm. of precipitated gossypol. It ate an equivalent of 0.3 gm. of the precipitated gossypol by the second day and seemed slightly indisposed. A week later it was again put on this feed, at the rate of 0.2 gm. daily. The quantity of gossypol eaten in the first six days was, per day, 0.2, 0.2, 0.17, 0.10, 0, and 0.05 gm. It ate none after this, but became sicker and died six days later.

Rabbit 961 ate 0.9 gm. of precipitated gossypol mixed with corn meal and molasses. It was apparently normal the next day, but refused cabbage on the third day. Thereafter it ate green feed well, but seemed to have no appetite for corn meal and molasses except when very hungry.

A week after recovery it was started on feed 319 (precipitated gossypol on corn meal). We planned to have it eat 0.3 gm. of gossypol daily. The first week 0.38 gm. of precipitated gossypol was eaten, the second week 0.67 gm., and only 0.60 gm. thereafter, a total of 1.65 gm. Death ensued after 19 days. The animal ate feed 319 sparingly and very irregularly.

A young rabbit (962) was fed similarly at the rate of 0.14 gm. a day. By weeks it ate, respectively, 0.97, 0.15, 0.15, and 0.34 gm. of precipitated gossypol. It was normal after the first week and died on the twenty-ninth day.

A guinea pig refused to do anything more than nibble feed 318 (gossypol extract), eating in 29 days only 34 gm. of the feed. It could not be induced to eat feed 319 (precipitated gossypol) any better, consuming only 1.13 gm. in 27 days. The autopsy showed that a mesenteric twist had cut off the blood supply of the last half or third of the intestines, so that death was not directly traceable to the feed.

Rabbit 949 was fed a large dose (1.44 gm.) of the precipitated gossypol mixed with corn meal and molasses. The next two days it suffered from diarrhea and refused to eat this feed, but it ate green feed. Thereafter it was given precipitated gossypol in small doses, but it usually refused all or part of this. Steadily losing weight, the animal died after 35 days, having eaten a total of 4.47 gm. of gossypol, inclusive of the large dose. The amounts eaten each week were, respectively, 2.08, 0.58, 0.50, and 0.68 gm.

Rabbit 937 had previously eaten the ether-extracted residue (feed 316) for 61 days and had increased in weight. Then, after several days on corn meal and molasses the rabbit was fed precipitated gossypol. We planned to feed 0.3 gm. a day, but only on three days did it eat this amount, usually refusing it entirely or in part. After 21 days a crystalline product was substituted for precipitated gossypol. The animal steadily decreased in weight and died after 33 days. The total amount of gossypol consumed was 2.52 gm. By weeks, 1.19, 0.27, 0.5, 0.57, and 0 gm. of gossypol were consumed. It ate practically nothing during the last 8 days. See Table V.

TABLE V.—*Results of feeding precipitated gossypol with corn meal and molasses (feed 319) to rabbits and guinea pigs*

Animal No.	Weight of animal.			Weight of precipitated gossypol eaten.	Number of days fed.	Result.
	Initial.	Final.	Loss.			
	Gm.	Gm.	Gm.			
Rabbit 954.....	1,740	1,275	465	1.02	13	Died.
Rabbit 961.....	1,830	1,435	395	1.22	19	Do.
Rabbit 962.....	630	465	165	1.62	29	Do.
Guinea pig A.....	660	565	95	1.13	27	Do.
Rabbit 949.....	2,375	1,702	673	^a 4.47	35	Do.
Rabbit 937.....	2,890	1,925	965	2.50	33	Do.

^a This quantity (4.47 gm.) includes a large dose of 1.44 gm. which evidently passed the bowel quickly.

POST-MORTEM OBSERVATIONS

Rabbit 954.—Excess fluid in abdominal cavity. Serous membrane in icteric condition.

Rabbit 961.—Large excess abdominal fluid. Small intestines show enteritis. Blood vessels congested.

Rabbit 962.—Large excess abdominal fluid. Small intestines inflamed and hemorrhagic. Small thrombus in right heart.

Guinea pig A.—Evidently died from mesenteric twist (convolvulus) in intestines. Posterior third greatly inflamed. Lungs congested and edematous.

Rabbit 949.—Slight excess of abdominal fluid. Small intestines conspicuously inflamed. Large pericardial abscess present. Enteritis.

Rabbit 937.—Slight excess abdominal fluid. Small intestines irritated throughout. Conspicuous thrombi in heart. Lungs congested and edematous.

TOXICITY OF CRYSTALLINE GOSSYPOL "ACETATE"

Crystalline gossypol "acetate" was obtained from a gossypol extract by the action of glacial acetic acid, which caused a slow deposition of yellow crystals. We have designated this substance as an "acetate," although the acetic acid present is not firmly bound.¹ The product corresponded in general properties to Marchlewski's gossypol. It was administered intraperitoneally to four rabbits, proving fatal, and was fed daily to eight rabbits. It made all of them sick. One died from secondary causes. Two refused to eat the feed after 5 and 15 days, respectively, and five died within from 13 to 55 days, having eaten from 0.35 to 2.53 gm. of crystalline gossypol "acetate."

INTRAPERITONEAL INJECTION OF CRYSTALLINE GOSSYPOL "ACETATE"

We dissolved 1.2 gm. of gossypol "acetate" in ether and mixed the solution with 16 c. c. of cottonseed oil. The ether was evaporated by heating over a steam bath. This was given intraperitoneally to two rabbits of about 1,100 gm. weight so that each rabbit received from 0.5 to 0.55 gm. of gossypol "acetate." Both animals died and were cold in six hours. The autopsy showed a considerable portion of the dose in the abdominal cavity, so that much more than a lethal dose was given.

About 3 gm. of a yellow, crudely crystalline product similar to that which was injected in 0.5 gm. doses to rabbits 955 and 956 was recrystallized as follows: The material was dissolved in hot alcohol and heated to boiling, then 50 per cent of acetic acid was added until the liquid became slightly turbid. This mixture was again heated to the boiling point and allowed to cool. Most of the substance separated in yellow, flat, pointed crystals, about 0.1 to 0.5 mm. long, which melted with darkening at about 178° C.

¹ The term "acetate" is arbitrarily used. Gossypol crystallizes from glacial acetic acid and even from quite dilute acetic acid with a molecule of acetic acid, which is not removed by long boiling with water or by heating to 115° to 120°. Its presence thus escaped our attention as it did Marchlewski's. It is entirely improbable that a small amount of acetic acid modifies in any way the physiological action of gossypol. See "Results of feeding precipitated gossypol."

To prepare the injection, 0.7 gm. of this substance was dissolved in ether and the ethereal solution mixed with 20 c. c. of purified cottonseed oil. The clear reddish yellow solution was warmed over steam until it had not the slightest odor of ether. This was then injected in doses of 10 c. c. into two rabbits, 963 and 964, weighing 1,560 and 1,485 gm., respectively. In a few minutes the rabbits became very uneasy and then passed into a sort of stupor. Rabbit 963 died in 3.5 hours and 964 in 4.5 hours. The death of rabbit 964 was witnessed. Shortly before death it toppled over on its side, had several convulsions, gasped several times, squealed, and died.

In these cases, as in the previous one, there was considerable injecta left in the abdominal cavity. See Table VI.

TABLE VI.—*Result of administering crystalline gossypol "acetate" intraperitoneally in cottonseed oil to rabbits*

CRYSTALLINE GOSSYPOL "ACETATE"

Rabbit No.	Initial weight of rabbit.	Weight of gossypol.	Dose volume.	Weight of gossypol per kilo of body weight.	Result.
	Gm.	Gm.	C. c.	Gm.	
955.....	1,115	0.55	8	0.493	Died.
956.....	1,180	.55	8	.466	Do.

RECRYSTALLIZED GOSSYPOL "ACETATE"

963.....	1,560	0.35	10	0.244	Died.
964.....	1,485	.35	10	.235	Do.

POST-MORTEM OBSERVATIONS

Rabbits 955 and 956.—Dead and cold after six hours. Apparent nonabsorption of much of the injection. Excess of fluid. Peritoneum stained brown. Visceral blood vessels slightly injected.

Rabbit 963.—Died in convulsions. Part of injecta present as oily globules. Serum present also. Serous membrane stained yellow.

Rabbit 964.—Same as 963, except small intestines were rather markedly injected.

FEEDING CRYSTALLINE GOSSYPOL "ACETATE" TO RABBITS

Crystalline gossypol "acetate" with corn meal and molasses (feed 319) was fed to rabbit 965. The feed was refused on the fourth day, after which it was not further given. Only on the first day did the animal eat the entire amount fed. After eating 0.3 gm. of crystallized gossypol "acetate," it had a bad diarrhea and little appetite for green feed the next day. The protocol was as follows:

December 15, first day.—Ate 0.3 gm. with corn meal and molasses; weight, 2,340 gm.

December 16, second day.—Bad diarrhea, and eats little green feed.

December 16, p. m.—Ate 0.2 gm. of gossypol.

December 17, a. m.—Ate green feed well.

December 17, p. m.—Ate 0.17 gm. of gossypol.

December 18, a. m.—Ate green feed well.

December 18, p. m.—Refused to eat the "doped" food.

December 19, a. m.—Slightly sick; eats green feed moderately.

December 19, p. m.—Refused to eat corn meal and molasses, but ate green feed.

Amount eaten, 0.67 gm.; final weight, 2,140 gm.; loss, 200 gm.

December 20 to December 31.—Ate green and corn meal and molasses; regained normal health.

Rabbit 951, weight 1,800 gm., which had previously stood two long feeding periods on ether-extracted cottonseed kernels, was fed crystalline gossypol "acetate." It ate 0.6 gm. in the first four days and then became sick, refusing all feed. On the tenth day it weighed 1,605 gm. From then till the twenty-eighth day, on which it died, it ate 0.32 gm. Weight about 1,170 gm.

Post-mortem observations: Teaspoonful excess in abdomen. Moderate injection of serous membranes. Some hemorrhagic areas in stomach. Mesenteric blood vessels more or less injected. Small thrombus in heart.

Rabbit 965A ate the same preparation of gossypol mixed with corn meal and molasses. It ate, by weeks, 0.64, 0.08, 0.5, 0.37, 0.07, 0.80, 0.31, and 0 gm.; total, 2.53 gm. This was a large healthy rabbit at the beginning. The post-mortem examination showed a slight excess of fluid in the abdominal cavity and serous membranes highly congested.

A new lot of rabbits was secured from a supply house in Washington, D. C. These rabbits were not as healthy and resistant as could be desired, some evidently having been used before in experimental work.

Rabbits 974, 976, 977, and 972 were given the same gossypol feed. Rabbit 974 ate 0.33 gm. of gossypol with corn meal and molasses. The next day it had a very bad diarrhea, which continued all day. It ate no green feed and only a little gossypol feed for the next four days, after which gossypol was withdrawn from the feed. On the nineteenth day it had not entirely recovered from the effects of eating 0.47 gm. of gossypol during the first five days. Loss in weight during 15 days, 330 gm.

Rabbit 976 was fed 0.25 gm. of gossypol. It had diarrhea the next day and no appetite. The third day it ate 0.05 gm.; fourth day, 0.09 gm.; fifth day, 0.01 gm.; and afterwards refused the gossypol feed. It lost in weight steadily until death, on the fifteenth day. Gossypol eaten, 0.40 gm. Loss in weight, 475 gm.

Rabbit 977 was fed like 976, with approximately the same effect. It died on the thirteenth day. Gossypol eaten, 0.35 gm. Loss in weight, 580 gm.

Rabbit 972 ate 0.55 gm. of gossypol and died in 13 days.

These last three rabbits were fed on a product which was somewhat darker in color than the gossypol given rabbit 974. The gossypol tends to take on a greenish or brown tinge under some conditions of prepara-

tion. Gossypol from old seeds is greenish. The post-mortem examination showed considerable irritation in the small intestines of these rabbits.

EFFECT OF SMALL DOSES OF CRYSTALLINE GOSSYPOL "ACETATE"

Rabbit 978, weight 2,100 gm., was fed on recrystallized gossypol "acetate" at the rate of 0.05 gm. daily mixed with corn meal and molasses. After one week it began to show a diminished appetite for the feed. On the nineteenth day it was given a double dose by mistake, and for two weeks thereafter showed a very poor appetite for the feed. At that time it weighed 1,820 gm.

Its record by weeks is as follows:

Quantity of gossypol eaten.....gm.. 0.34; 0.33; 0.335; 0.25; 0.25; 0.32;
0.125.

Weight of rabbit.....gm.. 2,045; 2,010; 2,070; 1,820; 1,930;
1,730.

Total quantity of gossypol eaten.....gm.. 1.95.

The animal died after 51 days. The post-mortem examination showed that a convolvulus had set up a necrotic condition in the intestine. Whether the feed was contributory to this condition we are unable to say. See Table VII.

TABLE VII.—Results of feeding crystalline gossypol "acetate" (feed 319) to rabbits

Feed and rabbit No.	Weight of rabbit.			Weight of gossypol "acetate" eaten.	Number of days fed.	Result.
	Initial.	Final.	Loss.			
Gossypol "acetate":	Gm.	Gm.	Gm.	Gm.		
965.....	2,340	2,140	200	0.67	5	Made sick.
951.....	1,800	1,170	630	.92	28	Died.
965A.....	2,265	1,600	665	2.53	55	Do.
Recrystallized gossypol "acetate":						
974.....	1,670	1,340	330	.47	15	Made sick.
976.....	1,670	1,195	475	.40	15	Died.
977.....	1,825	1,245	580	.35	13	Do.
972.....	1,425	1,115	310	.55	13	Do.
Gossypol "acetate" fed in small doses (0.05 gm. per day):						
978.....	2,100	1,730	380	1.95	51	Died from secondary causes.

FEEDING GOSSYPOL "ACETATE" TO FOWLS ¹

Two cockerels (986 and 987) previously fed on cottonseed meal to study the symptoms, were started on gossypol. Powdered gossypol in 0.3 gm. doses was fed, followed by a little water. On the fourth

¹ This experiment was carried on under the supervision of Dr. B. F. Kaupp, Pathologist, of the Poultry Division, North Carolina Agricultural Experiment Station.

day cockerel 986 had fallen off in weight, and his appetite was only fairly good. On the sixth day his digestion was poor, his crop being full of food. The bird steadily lost in weight until death on the sixteenth day, dropping from 3 to 2 pounds in weight. The bird was given 4.1 gm. of gossypol, at least 0.5 gm. of which was found in the crop after death.

The post-mortem examination showed extreme emaciation. Food in crop for a number of days; indications that gossypol interferes with the nervous mechanism of digestion. Diarrhea, the contents being fluid in rectum only. Semisolid in other portions. An absence of visible lesions.

Cockerel 987, slightly larger than 986, reacted in quite the same manner as 986 to administrations of gossypol. He steadily wasted away, falling from 3 pounds 8 ounces to 2 pounds 3 ounces, and died on the twenty-sixth day. Amount of gossypol fed, 5 gm.

The post-mortem examination showed extreme emaciation. Testes, spleen, gizzard, and other organs to a certain extent in a state of absorption.

Of chief interest to us was a statement by Dr. Kaupp to the effect that the gossypol produced the same results as cottonseed meal.

A healthy pullet (989) was started on gossypol. On the fourth day her digestion was affected. Nine doses of 0.3 gm. each in a period of 20 days were sufficient to cause her to refuse all feed and to waste away. She died on the thirty-sixth day, weight 1.5 pounds, just half the initial weight. Dr. Kaupp reported that "the autopsy revealed nothing beyond extreme emaciation." See Table VIII.

TABLE VIII.—Results of feeding gossypol "acetate" to fowls

Fowl No.	Weight of fowl.			Weight of gossypol "acetate" eaten.	Death occurred in—
	Initial.	Final.	Loss.		
	Pounds.	Pounds.	Pounds.	Gm.	Days.
986.....	3.0	2.0	1.0	4.6	16
987.....	3.5	2.2	1.3	5.0	26
989.....	3.0	1.5	1.5	2.7	36

FEEDING GOSSYPOL "ACETATE" TO A PIG

Pig 989, weighing 21 pounds, was fed corn meal and molasses. He ate with relish. About 5 p. m. he was given 3 gm. of crystalline gossypol "acetate" mixed with 80 gm. of corn meal and molasses, the whole feed weighing about 125 gm. He ate all but a small part. The next morning he had little appetite. In the afternoon he was given 1 gm. of gossypol on corn meal and molasses, most of which was left on the following morning. The remainder was made into slop. He ate part of this. On the afternoon of the same day he vomited; the following morning he appeared sick. We were unable to continue this experiment.

TOXICITY OF GOSSYPOL EXTRACT FREED OF GOSSYPOL (FEED 340)

Gossypol extract was treated with acetic acid for the preparation of gossypol "acetate," as previously described. The precipitate contained most of the gossypol. The filtrate, which contained only a small amount of it, was mixed with corn meal and dried. The extract, thus practically freed of gossypol, was fed to two rabbits in very large amounts and produced no symptoms of poisoning in either.

The rabbits weighed 1,995 and 1,986 gm., respectively. Each was fed the extract from 500 gm. of kernels during five days, the daily amounts for the first two days corresponding to 130 gm. each and for the three other days, 90 gm. each. No rabbit could have eaten within this short period without fatal results such a large amount of kernels or the gossypol from them.

TOXICITY OF OXIDIZED GOSSYPOL (FEED 338)

Withers and Ray (1913b) noted that the toxicity of cottonseed meal could be destroyed by boiling with alcoholic caustic soda. The alkaline alcoholic filtrate from this treatment was also found to be nontoxic, owing to the oxidation of the phenolic gossypol to an organic acid. To ascertain the correctness of this view, weighed amounts of recrystallized gossypol dissolved in alcohol were treated with dilute caustic soda. The solution was exposed to air overnight, made slightly acid with hydrochloric acid, and evaporated to dryness. The residue was mixed with corn meal and molasses for feeding. The substance had a pronounced bitter taste. Two small rabbits ate the oxidation product, equivalent to 3 gm. of gossypol apiece, in the course of 16 days without the slightest sign of being affected thereby. See Table IX.

TABLE IX.—*Result of feeding oxidized gossypol to rabbits*^a

Rabbit No.	Weight of rabbit.			Equivalent in gossypol of feed eaten.
	Second day.	Fifteenth day.	Gain.	
	Gm.	Gm.	Gm.	Gm.
983.....	1,280	1,420	160	3
984.....	850	1,065	215	3

^a On 4 days out of the 16 oxidized gossypol was not fed.

TOXICITY OF KERNELS WITH GOSSYPOL INCOMPLETELY EXTRACTED

ETHER-EXTRACTED KERNELS (FEED 316)

Decorticated cotton seeds were secured from Charlotte, N. C. They were sifted to remove as much lint and hulls as possible. The kernels were then ground in a mill and sifted through an 18- to 20-mesh sieve and

extracted for five to eight hours with ethyl ether in a filter-paper thimble in a large Soxhlet apparatus.^a After extraction the residual ether was evaporated and the kernels sifted through a 1-mm. sieve. They were either heated for an hour or so over a steam bath or dried in the air.

Sixteen rabbits and two guinea pigs were fed upon ether-extracted kernels. One of the rabbits had its back broken on the fifteenth day and was chloroformed. It showed none of the usual symptoms of cottonseed-meal feeding. Nine of the animals (Table X, part 1) died in from 19 to 75 days and 8 (Table X, part 3) were alive and normal at the end of the feeding experiments, which ranged from 42 to 71 days. Calculated to the average daily equivalent of kernels per kilogram of initial live weight, 9.4 gm. of ether-extracted kernels proved lethal to nine rabbits after 45 days, while 11.5 gm. did not prove lethal to eight others after 52 days. The ether-extracted kernels are therefore much lower in toxicity than cotton seed meal, of which a daily feed of 6.5 gm. per kilogram for 13 days was found lethal by Withers and Brewster (1913).

In view of the strikingly positive results obtained with ether extract and gossypol isolated therefrom, it was naturally expected that the ether-extracted kernels would prove nontoxic. With death resulting to only 9 out of 17 animals, and then not until after an average of 45 days, it is not unlikely that if the ether-extracted kernels had been fed in as small quantities as the cottonseed meal (6.5 instead of 9.4 gm.) they would have proved practically nontoxic, as anticipated.

The thoroughness of extraction is very important, as shown by the fact that kernels through which ether had only percolated proved toxic in from 11 to 14 days (Table X, part 2), while the average lethal period for kernels extracted from five to eight hours (Table X, part 1) was 45 days, or more than three times as long (Table X).

TABLE X.—Results of feeding ether-extracted cottonseed kernels (feed 316) to rabbits

PART I

Animal No.	Weight of rabbit.			Weight of feed eaten.	Equivalent of feed eaten as kernels.		Number of days fed.	Result.
	Initial.	Final.	Gain or loss.		Total.	Daily.		
	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.		
940.....	2, 100	1, 655	-455	348	522	14	35	Died.
935.....	1, 380	1, 500	+120	568	852	11	75	Do.
952.....	1, 480	1, 678	+198	251	373	20	19	Do.
947.....	1, 875	1, 576	-300	605	909	17	53	Do.
958.....	1, 430	1, 318	-112	494	741	15	52	Do.
959.....	2, 560	2, 165	-395	882	1, 323	21	64	Do.
Guinea pig B (second period) ^b .	610	535	-85	199	300	9	34	Do.
970.....	1, 515	1, 670	+155	285	420	21	20	Do.
974.....	1, 510	1, 525	+15	458	687	14	50	Do.

^a Before the ether extraction the ground kernels were extracted with petroleum ether or gasoline in case it was desired to work up the ether extract for gossypol.

^b Rabbit 651 and guinea pig B were fed for two separate periods, there being a rest of two weeks between the two periods.

TABLE X.—Results of feeding ether-extracted cotton seed kernels (feed 316) to rabbits—Continued

PART 2

Animal No.	Weight of rabbit.			Weight of feed eaten.	Equivalent of feed eaten as kernels.		Number of days fed.	Result.
	Initial.	Final.	Gain or loss.		Total.	Daily.		
	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.		
939.....	1, 510	1, 255	-255	^a 89	133	12	11	Died.
938.....	1, 930	1, 405	-525	^a 70	105	8	13	Do.
936.....	1, 415	955	-460	^a 69	103	7	14	Do.

PART 3

937.....	2, 630	2, 900	+270	1, 013	1, 520	25	61	Lived.
951 (first period) ^b	1, 800	1, 990	+190	609	914	22	42	Do.
951 (second period) ^b	1, 940	1, 790	-150	539	810	15	53	Do.
957.....	1, 650	1, 835	+185	627	942	21	46	Do.
Guinea pig B (first period) ^b ...	620	685	+ 65	320	480	10	50	Do.
960.....	2, 040	2, 195	+155	198	300	20	15	Chloroformed.
969.....	1, 475	1, 797	+322	798	1, 197	16	71	Lived.
981.....	1, 890	2, 230	+340	880	1, 320	30	44	Do.
985.....	1, 315	1, 730	+415	615	923	18	51	Do.

^a Feed percolated only with ether.^b Rabbit 951 and guinea pig B were fed for two separate periods, there being a rest of two weeks between the two periods.

Rabbits 939, 938, 936, and 940.—The post-mortem examination showed symptoms resembling cottonseed-meal poisoning.

Rabbit 935.—When this animal had recovered from the effects of the incompletely extracted kernels, it weighed 1,030 gm. It ate 647 gm. in 49 days and then weighed 1,600 gm. It died on the seventy-fifth day, showing symptoms other than those common to cottonseed-meal poisoning.

Rabbit 937.—Slightly off its feed in the middle of the experiment, but was in perfect condition when this feed was discontinued.

Rabbit 952.—Post-mortem examination showed a small amount of excess abdominal fluid and the small intestines considerably congested. Death due to enteritis. Symptoms of cottonseed-meal poisoning.

Rabbit 951 (first period).—Kept in good condition most of the time.

Rabbit 951 (second period).—Somewhat affected by feed. Ate but lightly at end.

Rabbit 947.—About 40 c. c. excess serous fluid in abdominal cavity. Considerable necrosis had set up.

Rabbit 957.—Perfectly well at the end of the experiment and remained so during the subsequent three weeks. It acquired no "immunity" toward cottonseed poisoning, however. See data on rabbit 957 on feed 290.

Rabbit 958.—Put on this feed after being made sick on unextracted kernels (feed 290). Post-mortem examination showed about 15 c. c. excess serous fluid in abdomen; small intestines markedly injected with slight hemorrhagic areas; liver congested; large abscess in submaxillary lymphatic glands.

Rabbit 959.—Began to be affected on the forty-seventh day, having gained up to this date. The post-mortem examination showed excess bloody serum in abdominal cavity; large amount of serum present with coagulated fibrin; serous membranes congested.

Guinea pig B.—In perfectly normal health at the end of first feeding period. Died in second experiment, showing much irritation in intestines.

Rabbit 960.—Broke its back accidentally and was chloroformed. Its case is of interest in that the autopsy showed no pathological lesions in the time usually required to kill an animal with cottonseed meal.

Rabbits 969 and 970.—Had previously been on the alcoholic extract (feed 330) for 26 days without ill effects.

ARE THE BAD EFFECTS OF FEED 316 DUE TO GOSSYPOL?

Feed 316 is of a pale-yellow color. Moistened with ether and examined through a lens, numerous black specks are seen, as in the unextracted kernels. These represent the gossypol glands, the contents of which have in part been removed by ether. Sometimes these glands have become separated from the seed tissue and can be examined individually. They dissolve in concentrated sulphuric acid with a red color, indicating gossypol. On warming a gram or so of the extracted kernels with alcoholic potash and shaking, a darkening in shade with a suggestion of a purple color takes place in the supernatant liquid. This is characteristic of gossypol, the depth of color depending upon the amount of gossypol. When the alcoholic alkali first touches the particles, they turn several shades deeper to a yellow that matches the color of cottonseed meal very closely. This is also characteristic of gossypol. On the addition of acid the former light-yellow color returns.

If the extracted kernels are allowed to soak in water for a short while, a substance dissolves which gives the liquid a reddish violet color. This is probably due to an oxidation product of gossypol. The coloration is quite permanent.¹

These experiments show that gossypol or oxidation products of gossypol or possibly other similar substances (see Power and Browning, 1914, p. 420) are still present in this residue after the long-continued ether extraction.

The fact that gossypol is not completely extracted by ether, although very soluble in it, may be due to its being held mechanically in impervious cells, being fixed dye like in the tissue, or being in the form of an insoluble metallic salt.

Therefore, it seems to us that even the slight toxicity of the residue after ether extraction is due to its gossypol content. (See data on rabbit 978, Table VII, p. 276.)

TOXICITY OF KERNELS PRACTICALLY GOSSYPOL-FREE

ETHER-ALCOHOL-EXTRACTED KERNELS (FEED 331)

In order to determine whether it were possible by extraction with solvents to prepare a cottonseed feed which would not produce any bad results with rabbits, the ground kernels were extracted first with gasoline to remove oil, etc., then with ether in a large separatory funnel until the percolate was of a very faint-yellow color. The residue was

¹ An attempt will be made to correlate this observation with the red sap (anthocyan?) of certain species of *Gossypium*.

removed and boiled in a large flask with alcohol. The first alcoholic extracts were quite highly colored. The extraction was repeated until a filtrate was obtained which possessed only a pale-yellow color.

The ether-alcohol-extracted kernels were fed daily to three rabbits for from 72 to 105 days in amounts ranging from the equivalent of 15.2 to 24 gm. of kernels; at the end of the period the rabbits were normal and all had gained from 30 to 148 per cent of their initial weight and were still gaining.

The severe test that these rabbits endured is sufficient to show that a feed has been prepared which can be called practically nontoxic.

It also indicates that protein and organic phosphates (inosite phosphoric acid salts), which are present in the feed in larger amounts than in cottonseed meal, have very little, if anything, to do with cottonseed-meal poisoning.

TABLE XI.—Results of feeding cottonseed kernels extracted with gasoline, ether, and alcohol (feed 331) to rabbits

Rabbit No.	Weight of rabbit.			Weight of feed eaten.	Equivalent of feed eaten as kernels.		Number of days fed.
	Initial.	Final.	Gain.		Total.	Daily.	
	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
966.....	1,335	1,897	562	1,043	1,738	24	72
967.....	640	1,590	950	957	1,595	15.2	105
968.....	1,610	2,095	485	1,108	1,846	19.6	94

Rabbit 967 was slightly off its feed only on the fortieth and forty-first days, but recovered quickly and continued to gain. See Table XII.

Rabbit 968 was one of the lot of Belgian hares received from Washington, D. C., in rather poor health. It was started at the rate of 15 gm. daily, equivalent to 25 gm. of whole kernels. This proved too heavy feeding, for after two weeks the animal went off its feed for several days. The ration was then reduced (Table XII).

TABLE XII.—Record of rabbits 967 and 968 on feed 331

Rabbit No. and period (10-day).	Weight of feed eaten.	Weight of rabbit.	Rabbit No. and period (10-day).	Weight of feed eaten.	Weight of rabbit.
	Gm.	Gm.		Gm.	Gm.
Rabbit 967.....		640	Rabbit 968.....		1,610
1.....	62	705	1.....	140	1,525
2.....	70	845	2.....	85	a 1,410
3.....	73	930	3.....	81	a 1,460
4.....	93		4.....	100	a 1,640
5.....	61	a 1,055	5.....	100	a 1,725
6.....	96	a 1,200	6.....	107	a 1,900
7.....	100	a 1,335	7.....	135	a 2,105
8.....	100	a 1,420	8.....	150	a 2,130
9.....	122	a 1,515	9.....	150	a, b 1,890
10.....	120	a 1,560	Last 4 days...	60	2,095
Last 5 days...	60	a 1,590			

a 10 gm. of corn meal was added daily to the feed.

b Loss in weight was due to the delivery of seven young rabbits.

TOXICITY OF AN ALCOHOLIC EXTRACT OF GASOLINE-ETHER-EXTRACTED KERNELS (FEED 330)

The solution obtained by treating gasoline-ether-extracted cottonseed kernels with hot alcohol was evaporated to a small volume over a water bath. The extract was about 10 to 12 per cent of the kernels. As the solution was concentrated, it separated into a yellowish layer (probably chiefly raffinose) and a reddish black resinous layer. The concentrated solution was mixed with corn meal, dried, and pulverized. This feed had a yellow-brown color and a very bitter taste. It was fed to two rabbits (969 and 970) in amounts equivalent to 50 gm. of cottonseed daily. It did not prove to be toxic, although the rabbits lost slightly in weight and frequently left part of their feed, possibly on account of its bitter taste. On the fourth day of feeding a slight diarrhea was noticed in both animals. They were quite normal after having been on the feed for 26 days, when it was discontinued (Table XIV).

TABLE XIV.—*Results of feeding an alcoholic extract of gasoline-ether-extracted cottonseed kernels (feed 330) to rabbits*

Rabbit No.	Weight of rabbit.			Weight of feed eaten.	Equivalent of feed eaten as kernels	Number of days fed.	Result.
	Initial.	Final.	Loss.				
	Gm.	Gm.	Gm.	Gm.	Gm.		
969.....	1, 530	1, 475	55	243	1, 000	26	Lived.
970.....	1, 650	1, 515	135	214	900	26	Do.

These two animals were then fed on the material from which the extract was obtained (see feed 316).

The presence of some gossypol due to the incomplete extraction by ether doubtless causes the slight toxicity of feed 316.

The nontoxicity of feed 330 may be explained on the assumption that the gossypol, extracted from feed 316 by alcohol, undergoes oxidation during the process of extraction or evaporation. This point needs further study (see feed 338).

Both the alcoholic extract and oxidized gossypol possess a bitter taste, whereas gossypol and gossypol "acetate" are tasteless and odorless.

ARE OTHER TOXIC SUBSTANCES PRESENT?

Although the feeding experiments show that gossypol is very poisonous, produces symptoms of cottonseed-meal poisoning, and affords a satisfactory explanation of the toxic properties of cottonseed meal, we do not claim to have made a complete study of the cottonseed from the standpoint of toxicity. The following problems are still unsolved:

(1) To exactly what extent does gossypol occur in cottonseed—i. e., in the petroleum extract and in the ether-extracted residue—and is gossypol the only toxic substance of like nature in the gossypol extract?

(2) To what extent, if any, do other toxic substances not related to gossypol contribute to the total action of cottonseed meal—i. e., are decomposition products and toxic alkaloids present in cottonseed meal? In this connection it may be stated that Friemann (1909) found an unidentified alkaloid in cottonseed meal, which caused paralysis of exposed frogs' hearts. Werenskiold (1897) obtained from cottonseed meal an alkaloid for which he proposed the name "gossypein." He also found betain and cholin. Withers and Fraps (1901, p. 81) state:

Gossypein, if present in the sample tested, was present in very minute quantity. The filtrate from 363 grams cottonseed meal, ready for precipitation with phosphotungstic acid, was extracted with chloroform, and nitrogen was determined in the extract. It was equivalent to 0.008 per cent gossypein (calculated as cholin).

Withers and Ray (1913b) state:

No evidence was found of the presence of toxic alkaloids in the feed, or of hydrocyanic acid in the feed or in the bodies of animals dead from eating cottonseed meal.

The fact that many solvents acting on *cottonseed meal* failed to remove the toxic substance suggests the possibility that in the manufacture of cottonseed meal the gossypol in the glands is fixed dyelike in the tissue of the seed, so that solvents like ether, in which gossypol is easily soluble, do not completely extract it. Gossypin is said to dye wool and silk (proteid materials). (See p. 265.) Again, some of the glands may be made impervious to the action of solvents by the mucilaginous substance surrounding the secretion. As is well known, cottonseed contains a large amount of raffinose (4 to 6 per cent). In the manufacture of the meal—e. g., in steaming—this may be partly dissolved and subsequently a film of this sugar deposited on the particles of meal. These factors must be considered with reference to the nonremoval of gossypol from the meal by solvents.

It may be noted that every gram of extracted residue represents at least 1.5 gm. of kernels. A ration of 15 gm. per day means that the animal eats all the protein and practically all the phosphorus of 22.5 gm. of seeds.

The residue (feed 316) is rich in nitrogen and ash. The values of nitrogen, protein, sulphur, and phosphorus in the ground kernels, and in feeds 316 and 331 are given in Table XV.

TABLE XV.—Percentage of nitrogen, protein, sulphur, and phosphorus in ground cottonseed kernels and in feeds 316 and 331

Feed.	Nitrogen.	Protein.	Sulphur.	Phosphorus.
Ground kernels.....	5.24	32.7	0.40
Feed 316.....	8.6	53.7	.54	1.2
Feed 331.....	8.8	55.0

It is quite probable that the animal organism is able to take care of the large amount of proteins and phosphorus compounds, as may be inferred from the results of feed 331.

The latest published endeavor to ascribe the poisonous effects to a specific chemical substance was by Crawford (1910), whose experiments seemed to point to salts of pyrophosphoric acid.

The improbability of this conclusion was shown by Withers and Ray (1913a), of this Station, in feeding experiments. Cottonseed meal was extracted with ammonium citrate. This left an insignificant amount of phosphorus in the residue, which was almost as toxic as whole cottonseed meal.

Edgerton and Morris (1912) also conducted many feeding experiments with cottonseed and cottonseed meal. They fed sodium phosphate in large amounts and concluded that they had found "no evidence whatever to show that pyrophosphoric acid has anything to do with cottonseed-meal poisoning."

Rather (1912) also studied the phosphorus compounds of cottonseed meal and concluded that there was no evidence that the samples of cottonseed meal examined contained either pyrophosphoric acid or metaphosphoric acid. He also states (p. 16) that "the inorganic phosphorus (Forbes' method), in the samples of cottonseed meal examined was less than 5 per cent of the total phosphorus."

R. J. Anderson (1912, p. 5) isolated an inosite phosphoric acid very similar to phytic acid and made the following statement:

The organic phosphoric acid of cottonseed meal gives all the reactions previously attributed to the presence of pyro- and meta-phosphoric acids. But the question whether or not it is also the toxic principle in cottonseed meal remains unanswered. Preliminary experiments carried out with the acid obtained from the purified barium salt on rabbits are not conclusive. Given in 0.5 and 1 gram doses, both the free acid and its potassium salt produced strong symptoms of distress, but after a few hours the animal regained their normal appearance. Larger doses passed through the bowel in a very short time and no definite symptoms developed.

It is difficult to determine just what caused the toxicity of the preparations which were used in the experiments described by Crawford. It is evident that very impure substances were fed.

Since inosite phosphoric acids occur in numerous feeding stuffs other than cottonseed meal—e. g., wheat bran, corn, oats, barley—and since no suspicion of toxicity has occurred in these substances it seems highly improbable that the phosphoric acids in cottonseed meal have any significant action as toxic agents.

METHODS FOR REMOVING OR DIMINISHING THE TOXICITY OF COTTONSEED

Three methods have been proposed at the North Carolina Experiment Station and have been found effective for diminishing the toxicity of cottonseed kernels or cottonseed meal:

(1) Extraction of the kernels with ether (feed 316) or with ether and with alcohol (feed 331). By these methods gossypol is reduced to such a small amount that the residue is only slightly toxic (feed 316) or is nontoxic (feed 331).

(2) Treatment of the meal with an alcoholic solution of an alkali (Withers and Ray, 1913b). This treatment affords conditions for rapid oxidation, and oxidized gossypol has been found by us to be nontoxic (feed 338).

(3) Treatment of the meal with iron salts (Withers and Brewster, 1913) and Withers (1913). Treatment with iron salts is accompanied by some chemical action, as shown by the pronounced change in the color of the meal. The favorable physiological changes may be due to oxidation of the gossypol or to the formation of a more difficultly soluble compound. The oxidation may be due to the stimulating action of iron upon the oxidases of the animal body or to the direct action which ferric salts exert upon phenolic bodies. Ferrous sulphate forms an insoluble lake with gossypol. We have not yet studied it, but as Marchlewski (1899) found the lead salt so stable that it was not decomposed by hydrogen sulphid nor sulphuric acid, it is likely that the iron lake is very stable also.

The seed tissue surrounding the cells probably prevents the free action of reagents which would extract gossypol or render it physiologically inert. This constitutes the principal difficulty that must be overcome by the oil miller or stock feeder in rendering cottonseed meal nontoxic.

SUMMARY

(1) Gossypol, first isolated by Marchlewski from cottonseed oil and considered by him a prospective dyestuff, was extracted by us from cottonseed kernels and found to possess toxic properties.

(2) Cottonseed kernels were used as the initial material instead of cottonseed meal, because they yield gossypol more readily to solvents and are toxic to about the same extent.

(3) Ethyl ether was used as the solvent, the kernels having been extracted with gasoline to remove most of the oil. Evaporation of the ether leaves a crude product which we have designated "gossypol extract." A purer product, "precipitated gossypol," was obtained from the ethereal solution by the addition of gasoline, and a crystalline product, "gossypol 'acetate,'" by precipitation by acetic acid.

(4) Gossypol was fatal to rabbits when administered intraperitoneally in the form of gossypol extract or crystalline gossypol acetate, either when fed in one large dose in the form of gossypol extract or when fed in small daily doses in the form of gossypol extract, precipitated gossypol, or gossypol "acetate."

(5) Gossypol forms an oxidation product which is nontoxic.

(6) Cottonseed kernels are rendered less toxic by the partial extraction of gossypol and nontoxic by a more nearly complete extraction of it.

(7) Methods for rendering cottonseed kernels nontoxic depend upon extracting the gossypol or changing it to physiologically inert forms by oxidation or by precipitation.

(8) The smallest amount of gossypol administered intraperitoneally by us and found fatal to rabbits was 0.24 gm. of crystalline gossypol acetate per kilo of live weight.

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PLATE XXV

Gossypol glands of the cottonseed:

Fig. 1.—Lengthwise sections of cottonseed kernels, showing glands, folded cotyledons, and hypocotyl. $\times 8$.

Fig. 2.—Cross sections of five widely different varieties of cottonseed kernels: *a*, Russell Big Boll; *b*, Willet's Red Leaf; *c*, Piedmont Long-Staple; *d*, Allen's Early; *e*, Wine Sap. $\times 8$.



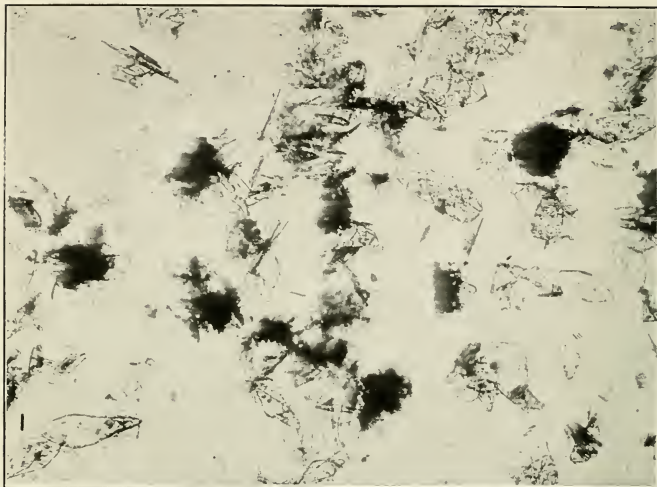


PLATE XXVI

Fig. 1.—Crystals of gossypol "acetate" from alcohol and 50 per cent acetic acid. $\times 25$.

Fig. 2.—Crystals of gossypol from acetone. $\times 25$.



TWO NEW HOSTS FOR PERIDERMIIUM PYRIFORME

By GEORGE GRANT HEDGCOCK, *Pathologist*, and WILLIAM H. LONG, *Forest Pathologist*,
Investigations in Forest Pathology, Bureau of Plant Industry

Peridermium pyriforme Peck, which is the æcial form of *Cronartium pyriforme* (Peck) Hedgec. and Long, was collected for the first time on *Pinus rigida* Mill. by the senior writer on June 16, 1915, near Essex Junction, Vt. (F. P. 17708).¹ This is the first collection which has been reported on this host. The senior writer had previously found the uredinial and telial forms in abundance in the same locality on *Comandra umbellata* (L.) Nutt. (F. P. 8655) on July 31, 1913. This find is important, since it may serve to clear up the mystery associated with the identity of the host in the case of the type specimen on *Pinus* spp.,² collected by Prof. J. B. Ellis (2040) in 1880, possibly near Newfield, N. J., Ellis not being certain as to the locality. Since the telial form was collected by Ellis (Ellis and Everhart, N. A. Fungi, No. 1082) near Newfield in 1879 and as *Pinus rigida* is the only native species of pine in this locality known to be attacked by the fungus, it is very probable that this species is the host of the type. In measurements and shape the spores of the writers' specimen agree with those of the type which the writers have examined at the herbarium of the State Museum at Albany, N. Y. The type specimen consists of a young pine twig whose bark closely resembles in color and markings that of *Pinus rigida*.

Mr. Roy G. Pierce, of this office, collected a number of specimens of *Peridermium pyriforme* on *Pinus divaricata* (Ait.) Du Mont de Cours (Pl. XXVII, fig. 1) in several localities near Cass Lake, Minn., during the month of June, 1915 (F. P. 18044, 18046, 18047, 18058, 18060, 18072, and 18076). So far as the writers know, only one specimen of the fungus has hitherto been reported on *Pinus divaricata*, and that was found by Mr. J. J. Davis in Douglas County, Wis. Mr. Pierce reported that the fungus was common where he collected it, and it is probably common also in other localities. He also found the uredinial form, *Cronartium pyriforme*, on July 11, 1915, on *Comandra umbellata* in the same locality as one of his previous collections of the æcial form.

The junior writer also has a specimen of this rust (F. P. 19440) on *Pinus divaricata* collected at Roscommon, Mich., by State Forester Marcus Schaaf. This specimen was sent in with *Peridermium cerebrum*, which on this host produces typical globular swellings, while *Peridermium pyriforme* causes the typical fusiform swellings. *Peridermium pyriforme*, however, does not always produce fusiform swellings, since the junior writer has recently received a specimen (F. P. 19437) on a 4-year-old

¹ "F. P." = Forest-Pathology Investigations number.

² Hedgecock, G. G., and Long, W. H. A disease of pines caused by *Cronartium pyriforme*. U. S. Dept. Agr. Bul. 247, p. 7. 1915.

transplant of *Pinus (murrayana) contorta* Loud., collected at Roscommon, Mich., by Mr. Schaaf, which produced a globoid gall (Pl. XXVII, fig. 2) extending nearly around the attacked stem. This gall was 6 cm. in circumference and 2 cm. in diameter. Both above and below the gall were irregular lesions caused by *Peridermium comptoniae* (Arthur) Orton and Adams. The gall resembled so closely the swelling produced by *Peridermium cerebrum* that the junior writer thought it was this species until he examined it under the microscope, when he found the typical pyriform spores of *Peridermium pyriforme*.

In June, 1915, the junior writer received a fine specimen of *Peridermium pyriforme* (F. P. 19429) on *Pinus arizonica* Engelm., a 3- to 5-leaved pine (Pl. XXVII, fig. 3), collected by Ranger J. H. Woolsey in Jacobson's Canyon, Crook National Forest, Arizona. This is the first time this rust has been reported on this host. Many of the æcia of the specimen were very large and unusually prominent, owing to their marked extension beyond the bark. Some were over 2 cm. long and from 5 to 6 mm. in height. The galls were of the effused type and were from 40 to 50 cm. long. One of the branches attacked was about 2 inches in diameter where the lesions occurred. Its bark was very rough and exfoliated by the action of the fungus. The lesions had completely surrounded the two branches for a distance of from 20 to 30 cm., but had not yet killed them.

The writers have previously found *Peridermium pyriforme* only on pines having two to three needles in the leaf cluster,² and the occurrence of the fungus as now reported on *Pinus rigida* and *Pinus arizonica* is of interest, since it adds to the list of known hosts two pines of the group bearing three needles in a cluster. *Pinus rigida* has three needles and *Pinus arizonica* three to five needles.

It is now known that *Peridermium pyriforme* causes three forms of disease on pines; one with slight or no hypertrophy, common on *Pinus divaricata*, *Pinus pungens* Michx., and *Pinus ponderosa scopulorum* Engelm.; a second causing a fusiform or spindle-shaped swelling and found on *Pinus arizonica*, *Pinus (murrayana) contorta*, *Pinus divaricata*, *Pinus ponderosa* Laws., *Pinus ponderosa scopulorum* Engelm., and *Pinus rigida*; and a third form, causing the formation of globose galls (Pl. XXVII, fig. 2) now first reported on *Pinus (murrayana) contorta*.

Peridermium pyriforme, especially when weathered, superficially resembles *Peridermium comptoniae*, with which the senior writer found it associated near Essex Junction, Vt., where he found 1 specimen of the former and nearly 50 of the latter species. It is quite probable that this resemblance has frequently caused it to be overlooked by collectors wherever two species occur together and that a more careful search for *Peridermium pyriforme* will greatly extend the known range of the disease of pines caused by it. The spheroid galls of *Peridermium pyriforme* resemble very closely the spheroid galls of *Peridermium cerebrum* (Pl. XXVII, fig. 2); and unless the spores are examined, this form might be easily mistaken for the latter fungus.

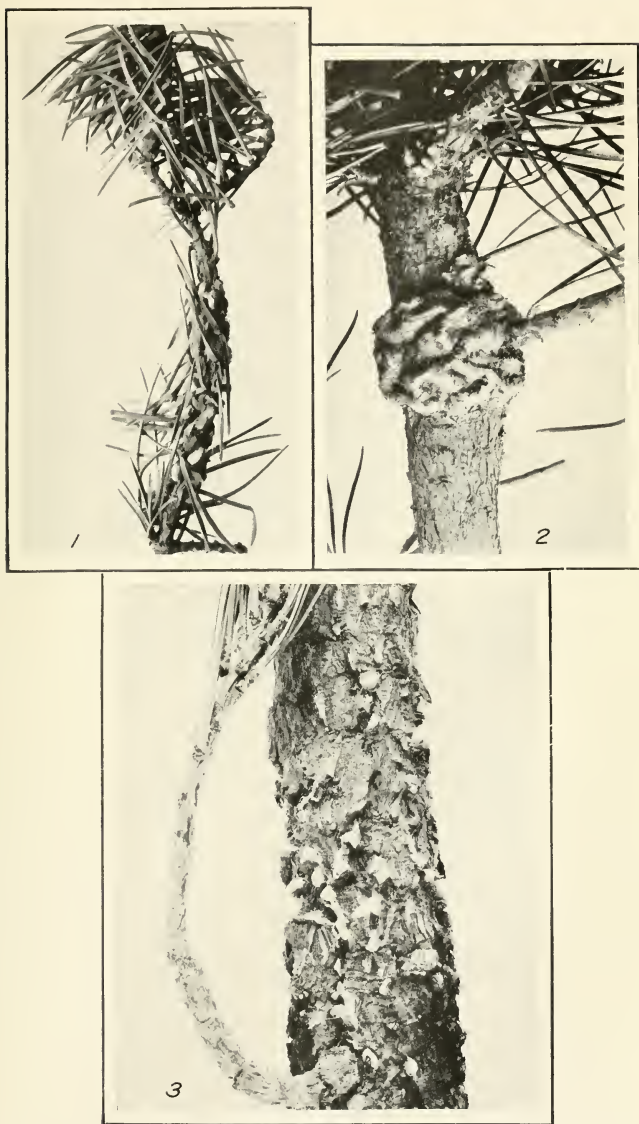
² Hedgcock, G. G., and Long, W. H. Op. cit.

PLATE XXVII

Fig. 1.—*Peridermium pyriforme* (F. P. 18044) on a trunk of *Pinus divaricata*, showing the form of the peridia before they are ruptured to allow the escape of the æciospores.

Fig. 2.—A globose gall with *Peridermium pyriforme* on a trunk of *Pinus contorta* (F. P. 19437), associated with two lesions of *Peridermium comptoniae*, one near the gall and the other 1 inch above it at the base of a branch.

Fig. 3.—*Peridermium pyriforme* (F. P. 19429) on a branch of *Pinus arizonica* showing unopened peridia. This branch was 1 inch in diameter and 10 years old.



PATHOGENICITY AND IDENTITY OF SCLEROTINIA LIBERTIANA AND SCLEROTINIA SMILACINA ON GINSENG

By J. ROSENBAUM,¹

*Mycologist, Cotton and Truck Disease Investigations,
Bureau of Plant Industry*

INTRODUCTION

For a number of years two species of *Sclerotinia* have been recognized as probable causes of the rotting of ginseng roots (*Panax quinquefolia*), but the pathogenicity and identity of these fungi have not been proved by by inoculation experiments.

The purpose of this paper is (1) to report inoculation experiments establishing the pathogenicity of these organisms, and (2) to detail the experimental data and considerations on which the conclusions as to the identity of the two pathogens are based.

WHITE-ROT OF GINSENG

The white-rot of ginseng was first reported by Whetzel (1907, p. 89).² *Sclerotia* were found, but the identity of the fungus was not determined. Subsequent workers, Rankin (1910), Osner (1911), and Whetzel and Rosenbaum (1912, p. 34-45) have attributed the disease to *Sclerotinia libertiana* Fuckel. These writers based their observations on the association of the sclerotia of the fungus with the host and the general resemblance of the organism on the host and in culture to the widespread *Sclerotinia libertiana*. No inoculation experiments have been reported.

PATHOGENICITY

During the spring of 1913 the fungus was isolated from diseased ginseng roots grown at Newtown, Pa., Mentor, Ohio, and Edenville, Mich. The isolations were made by washing the roots, immersing them for 10 minutes in a solution of mercuric chlorid (1 to 1,000), peeling back a portion of the external tissues, and transferring small bits of tissue from the inside of the root to poured plates of hard potato agar. Pure cultures were obtained in the majority of cases from the first planting. In addition to the cultures isolated from ginseng, inoculations on healthy ginseng

¹ The writer is indebted for many suggestions to Dr. Donald Reddick, of Cornell University, under whose direction this work was done.

² Bibliographic citations in parentheses refer to "Literature cited," p. 297.

roots were also made with a culture of *Sclerotinia libertiana* obtained from lettuce from South Carolina. The procedure followed in the inoculations was as follows: Healthy ginseng plants with the tops still attached were selected and the soil carefully removed from one side of the root. By means of a flamed scalpel longitudinal cuts were made in the side of the root. These cuts were approximately one-fourth of an inch in length and about one-eighth in depth. A piece of agar containing mycelium from young cultures was inserted within these cuts and covered with soil. Check roots were treated in a similar manner.

During the summer inoculations were made as shown in Table I. The checks in every case remained healthy.

TABLE I.—Results of the inoculation of ginseng with *Sclerotinia libertiana* from various sources

Date.	Source of culture.	Number of roots inoculated.	Number of checks.	Percentage of infection.
July 14	<i>Sclerotinia libertiana</i> from South Carolina from lettuce	6	2	100
15	<i>Sclerotinia</i> sp. from Mentor, Ohio, from ginseng.	6	2	100
15	<i>Sclerotinia</i> sp. from Newtown, Pa., from ginseng.	8	4	100
15	<i>Sclerotinia</i> sp. from Edenville, Mich., from ginseng	6	2	83+
Aug. 1	<i>Sclerotinia</i> sp. from Mentor, Ohio, from ginseng.	4	1	100
1	<i>Sclerotinia libertiana</i> from South Carolina from lettuce	4	1	75

Plate XXVIII, figures 1 and 2, is reproduced from photographs of ginseng roots from two of the above series. Figure 1 shows a root inoculated with *Sclerotinia libertiana* isolated from lettuce. Figure 2 shows three roots (on the left) inoculated with a species of *Sclerotinia* isolated from ginseng.

Reisolations were made from the inoculations of July 15 and the fungus was again grown in pure culture. Inoculations made with the reisolated culture gave positive results.

Infection was evident in from three to seven days after inoculation. The root at the point of inoculation becomes soft and the rot spreads gradually in all directions, causing the entire root to become soft and doughy. After the mycelium has penetrated throughout the tissues of the root, it forms tufts of cottony-white felt, in which large black sclerotia rapidly develop. Sclerotia on the outside of the root have in some cases developed within 10 days after the inoculations were made. When the inoculations are made near the crown of the root, the mycelium spreads to the stem, where it develops similar sclerotia on both the inside and the outside of the stem. The rapidity with which the disease progresses in the inoculated roots depends upon moisture conditions.

During a rainy period infection is evident within a much shorter time. All attempts to produce the disease without previously injuring the root gave negative results.

IDENTITY OF THE SPECIES

In order to further prove that the species of *Sclerotinia* from ginseng is identical with *Sclerotinia libertiana* Fuckel, a comparison was made with cultures from different sources. In addition to the four strains mentioned above, there was also used a pure culture isolated by Dr. Donald Reddick, of Cornell University, from celery. The comparison of the strains consisted in (1) growing the cultures on different media, both acid and alkaline; (2) production of apothecia, measurements of asci, ascospores, and a study of the manner of germination; (3) cross-inoculations on lettuce. These topics are briefly discussed in the following paragraphs.

GROWTH ON DIFFERENT MEDIA.—Cultures were made on potato agar, nutrient agar, bean plugs, ginseng stems, and Raulin's synthetic fluid. In the case of potato and nutrient agar both acid and alkaline media were used (± 10.5 Fuller's scale). On all the media the various strains made a good growth, but no differences were visible.

PRODUCTION OF APOTHECIA, ETC.—In order to obtain apothecia from the various strains, the sclerotia produced in pure culture were placed on sterile moist sand in dome-shaped preparation dishes. The sclerotia were covered with a very thin layer of the sand, and the dishes were placed on a shelf in front of a window. The time required for these apothecia to develop varied greatly, the limits being from three weeks to three months. The size of the apothecia likewise varied even in the case of sclerotia from the same strain and produced in the same test tube. However, the apothecia were alike in general appearance in all the strains. Plate XXVIII, figure 3, shows apothecia from the celery strain, and Plate XXVIII, figure 4, shows the same from the ginseng strain. A large number of measurements made of asci, paraphyses, and ascospores showed no marked variations, and agreed with the description of *Sclerotinia libertiana* Fuckel as given in Saccardo. In figure 1, A, is shown a camera-lucida drawing of asci, ascospores, and paraphyses from a fresh preparation of the Mentor strain.

Crushed pieces of apothecia were placed in drops of water in order to observe the ascospore germination. Within four hours after being placed in water the first signs of germination became visible. Figure 1, B, shows the ascospores within the asci, germinated by sending germ tubes directly through the walls of the ascus. No differences were noted in the germination of the spores from the different strains.

INOCULATIONS ON LETTUCE.—Mature lettuce plants were selected and inoculated with the various strains of the fungus. Inoculations were

made on injured and uninjured plants, which were then covered with bell jars for 4 days. At the end of 12 days most of the plants showed signs of rotting. Unlike the ginseng roots (Pl. XXVIII, figs. 1 and 2) previously discussed, infection occurred not only on the injured, but also on the uninjured plants.

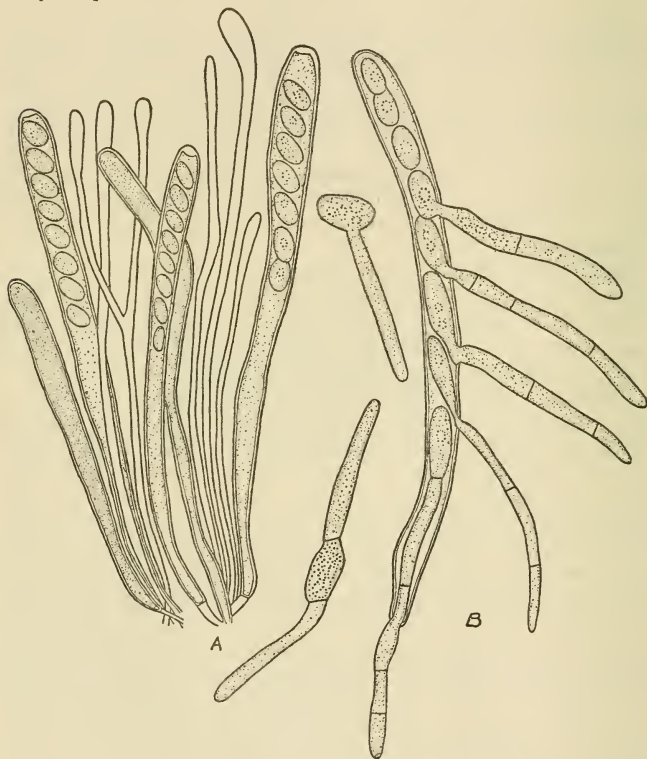


FIG. 1.—*Sclerotinia libertiana*: A, Camera-lucida drawing showing branched and unbranched paraphyses, asci, and ascospores; B, camera-lucida drawing showing methods of ascospore germination. Those within the asci germinate by sending germ tubes directly through the walls of the ascus.

BLACK-ROT OF GINSENG

Van Hook (1904, p. 181-182) first mentions a species of *Sclerotinia* as the cause of a black-rot of ginseng. Rankin (1912) reports the discovery of the apothecia and established a new specific name for the fungus. No inoculations were attempted, either on the ginseng roots or on other hosts known to be attacked by species of *Sclerotinia* closely allied to this one.

PATHOGENICITY

In the spring of 1912 the writer received a number of black-rotted roots from Wisconsin showing various stages of development of the disease. Isolations were made from these roots by making plantings from the inner tissues of the roots on poured plates of hard potato agar. The fungus was obtained in pure culture, where it produces a characteristic black growth.

Inoculations on healthy roots made at various times during the summer gave negative results, as would be expected from the nature of the fungus, since the disease always develops in beds during the winter. In October of the same year (1912) six roots were washed clean and inoculated by placing a piece of the agar pure culture in a small cut made in the tissues of the root. Three similar roots were injured and used as checks. All the roots were planted in soil which had never grown a crop of ginseng. The following March an examination of the roots showed the characteristic symptoms of the disease. Some were entirely black, while others were only partly blackened. The fungus was easily reisolated from these roots. Plate XXIX, figure 1, shows two inoculated roots, together with a check root. One of the inoculated roots is entirely black, while the second shows this black color only in part.

In October, 1913, inoculations were again made on ginseng roots. These roots were not injured, but the fungus was placed on the old stem scar. The next March the roots were black, as in the previous year. Reisolations were again made, and the fungus which was obtained produced the characteristic black growth.

IDENTITY OF THE SPECIES

The growth of the fungus in culture and the general behavior of this organism differed so greatly from the known species of *Sclerotinia* that it has always been an interesting question as to the source of the fungus which appeared in isolated gardens throughout the country. One plausible explanation is that the fungus, being associated with wild ginseng roots or with one of the common weeds, was brought in from the woods, as many growers make a practice of using leaf mold in preparing their beds. Since the fungus from the description resembled *Sclerotinia smilacina* Durand, it seemed advisable to determine whether the species of *Sclerotinia* on ginseng could produce a black-rot of the rhizome of *Smilacina* spp. and whether the two were also identical in other respects.

INOCULATIONS ON SPECIES OF SMILACINA.—In October, 1913, six rhizomes of *Smilacina racemosa* were inoculated with a pure culture of the black-rot fungus obtained from ginseng. The inoculations were made by slightly injuring the rhizome and inserting the mycelium of the fungus in the cut. Check plants were also injured. When examined the following March, the rhizomes showed the characteristic symptoms of black-rot

as exhibited by ginseng roots. The check plants remained healthy. Plate XXIX, figure 2, is a reproduction of a photograph of two of the inoculated and one check rhizome. Reisolations were made, and the fungus which was obtained resembled the original culture isolated from ginseng.

COMPARISON WITH TYPE SPECIMEN.—To determine further the relationship of the *Sclerotinia* sp. from ginseng to that on *Smilacina* spp., an examination was made of the type specimen of *Sclerotinia smilacina* Durand, deposited by Dr. Durand in the herbarium of the botany department of Cornell University. The specimens showed the black coloration as exhibited by the inoculated rhizomes of *Smilacina racemosa* as well as the ginseng roots.

Apothecia on ginseng are rare, and though attempts to produce them were made no success can be reported up to the present time. It is of interest, however, to compare the measurements as given in the original descriptions by Durand (1902, p. 462-463) and Rankin (1912) as shown in the following table:

Species.	Sclerotia.	Apothecia.	Asci.	Ascospores.
	Gm.	Gm.	μ	μ
<i>Sclerotinia smilacina</i> . .	0.1 by 0.2 to 2.	0.75 to 3. .	120 to 140 by 6 to 8.	12 to 15 by 4 to 5.
<i>Sclerotinia panacis</i> . . .	0.3 to 1.	1.5 to 2.5. .	125 to 137.5 by 6.4 to 6.5.	11.7 to 16 by 4.8 to 7.5.

Measurements made by the writer from the type material of these species have shown that the asci and ascospores are not to be distinguished either in form or size and agree with the measurements given above.

CONCLUSIONS

1. (A) The pathogenicity of *Sclerotinia* sp. causing the white-rot of ginseng has been established. (B) This species of *Sclerotinia* is identical with the *Sclerotinia libertiana* Fuckel occurring on lettuce, celery, and a number of other hosts.

2. (A) The pathogenicity of *Sclerotinia* sp. causing the black-rot of ginseng has been established. (B) A consideration of the following facts indicates that *Sclerotinia panacis* Rankin is identical with *Sclerotinia smilacina* Durand: (a) Inoculations with a species of *Sclerotinia* from ginseng on *Smilacina racemosa* gave positive results. (b) Measurements of asci and spores made by the writer from the type material of both species agree. There is a close agreement in all distinguishing characters, as given in the original description of the two species. (c) The lesions produced by the inoculations are similar on the two hosts and identical with those on diseased plants as they occur naturally.

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PLATE XXVIII

Sclerotinia libertiana:

Fig. 1.—Root inoculated with *Sclerotinia libertiana* from lettuce. Note the white mycelial felt and the production of sclerotia.

Fig. 2.—Three roots (on left) inoculated with *Sclerotinia* sp. from ginseng. Healthy check root (on right).

Fig. 3.—Apothecia from sclerotia from celery strain.

Fig. 4.—Apothecia from sclerotia from ginseng strain.

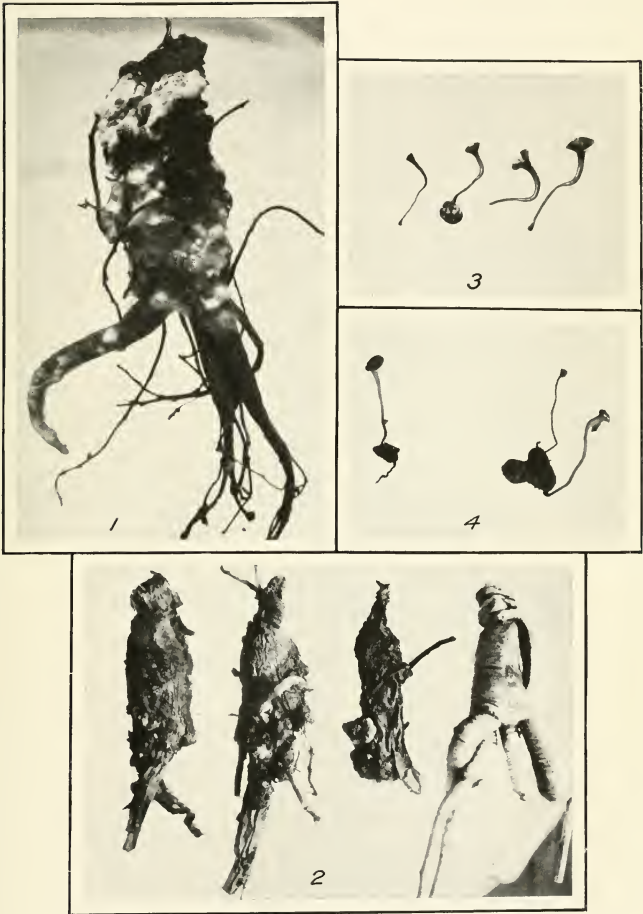




PLATE XXIX

Sclerotinia smilacina:

Fig. 1.—Ginseng roots showing the characteristic black color from artificial inoculation. The root on the left is the check.

Fig. 2.—Rhizomes of *Smilacina racemosa* inoculated with a species of *Sclerotinia* isolated from ginseng. The rhizome on the right is the check.

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AN IMPROVED RESPIRATION CALORIMETER FOR USE IN EXPERIMENTS WITH MAN

By C. F. LANGWORTHY, *Chief*, and R. D. MILNER, *Assistant Chief*,
Office of Home Economics, States Relations Service

INTRODUCTION

The nutrition of the human body consists mainly in the transformation of food into body material and the ultimate transformation of the energy potential in both food and body material into such forms of energy as heat and muscular work. The transformations of both food and body material occur largely in accordance with the needs of the body for energy. To understand the laws governing the nutrition of the body, knowledge regarding these transformations of matter and energy is essential.

To obtain such knowledge it is necessary to have some means of determining the intake and output of both matter and energy by the body. This involves the use of some form of apparatus that will give an accurate measurement of the gaseous exchange and the energy production of the body. Such an apparatus is the so-called respiration calorimeter employed in connection with the nutrition investigations of the Department of Agriculture.

The first apparatus of this kind constructed in this country was developed in connection with these investigations. Work on this device was begun in 1892 by Prof. W. O. Atwater at Wesleyan University, Middletown, Conn. When the Department of Agriculture undertook an inquiry into the food and nutrition of man in 1894 as a logical outgrowth of the earlier work of Prof. Atwater for the Smithsonian Institution and the United States Department of Labor, the need of some means of determining the income and outgo of matter and energy in the body was recognized, and the general plan of work to be undertaken as part of the inquiry was made to include experiments with the respiration calorimeter which had been devised for measuring factors of outgo.

For use in the study of the output of matter by the body, the device was similar in principle to the respiration apparatus of Pettenkofer (16),¹

¹ Reference is made by number to "Literature cited," p. 346-347.

with alteration in detail in accordance with modification in methods of investigation, but in its equipment for the measurement of the output of heat it was quite original. Prof. E. B. Rosa, then of Wesleyan University and associated with Prof. Atwater in the investigations, devised a method of preventing the passage of heat through the walls of the respiration chamber, and provided for carrying out and measuring the heat generated within it. The term "respiration calorimeter" was applied to the Atwater-Rosa device to indicate that it performed simultaneously the functions of both a respiration apparatus and a calorimeter.

Experiments with the respiration calorimeter have been continued as part of the nutrition investigations of the Department of Agriculture during the 20 years or more since they were begun. With the progress of the work many modifications have been introduced for the purpose of making the apparatus simpler, easier, and more economical to operate than the original, while yielding more complete and more accurate data. Descriptions of the apparatus in its original form and its later modifications, and the results of a large number of experiments with it, have appeared in former publications of the Department (1, 2, 3, 4, 6, 9) and have become a part of the data commonly included in textbooks and works of reference.

As a result of the work of Atwater and his associates, the investigator has been provided with an apparatus of precision and a method of investigation which, with adaptation in different laboratories to meet varied experimental conditions, have proved valuable for a range of work even wider than was originally anticipated. In the nutrition laboratories of the Department of Agriculture it has been employed in the form described in the present publication in studies of the utilization of food and the performance of muscular work, and a recent development, to be described in detail in a later publication, has been adapted to studies of problems in plant physiology. At the Institute of Animal Nutrition, State College, Pa., Dr. H. P. Armsby employs a respiration calorimeter, which he has adapted from the original Atwater-Rosa type of apparatus, in investigations of the nutrition of farm animals conducted in cooperation with the Department of Agriculture. In other inquiries besides those of the Department respiration calorimeters have proved of great value in investigations of different but related character. Investigators have modified and improved the original form to suit their special needs, though this method of research has long passed the experimental initial stage and has become recognized as possessing great possibilities where accurate measurements of energy values and gaseous exchange are needed to supplement the data which the investigator secures by other methods.

The respiration calorimeter employed at the present time in the nutrition investigations of the Department of Agriculture is a development of

the one used for over 12 years in the laboratory of Prof. Atwater. In 1907, when because of illness he discontinued his connection with the research, the respiration calorimeter was transferred to Washington. To move the apparatus it was necessary to dismantle it completely, so that to set it up again in the laboratory provided for it in the new building of the Department involved its practical reconstruction. Advantage was taken of the opportunity thus afforded to modify it in many important details, with special consideration for simplicity of structure and convenience of operation. The reconstructed apparatus has been briefly described in a former publication of the Department (15) and elsewhere (14). The experience with this apparatus has suggested further improvements that have been incorporated from time to time, with the result that the work of conducting an experiment with the respiration calorimeter is much less than formerly, and a degree of accuracy of measurement is obtained that was not possible with the apparatus in its earlier state. The present publication describes this greatly improved respiration calorimeter in detail. A general view of the apparatus is shown in Plate XXX.

PRINCIPLE OF THE RESPIRATION CALORIMETER

The principle of the respiration calorimeter now in use in the nutrition investigations is the same as that of the later form of the apparatus employed in the investigations formerly conducted at Wesleyan University. For the determination of gaseous exchange the device is similar to the respiration apparatus of Regnault and Reiset (17), having a respiration chamber and a system of air-purifying devices connected in series in a closed circuit. The air confined in the circuit is kept in circulation, the respiratory products imparted to it by the subject in the chamber being constantly removed and oxygen constantly supplied to replace that used by the subject. For the determination of heat produced in the chamber the device is a constant-temperature, continuous-flow, water calorimeter, in which the calorimetric features of the original Atwater-Rosa apparatus are retained. These provide for preventing the passage of heat through the walls of the chamber and for taking up the heat by a current of cold water as fast as it is generated in the chamber. The determination of respiratory exchange and energy transformation, to be of value, demands a high degree of accuracy in the fundamental measurements, and it follows that the instrument with which they are made must be precise and finely adjusted, sensitive to slight changes within, and protected from the effects of fluctuations occurring outside of it.

Of fundamental importance in the device is a chamber with walls that are air-tight and heatproof. It must be so large that the subject may live in it in comfort during the time of an experiment, which may continue several hours or several days, and yet not so large that its volume

will prevent the accurate measurement of the amounts of the different gases in the air inclosed. Its walls must be absolutely air-tight, because any leakage of air would nullify the determination of the respiratory exchange, and there must be no passage of heat through them, because any transference of unmeasured heat into or out of the chamber would introduce error into the determination of the amount of energy produced within it. In the following pages the construction of the chamber of the apparatus is described, and the auxiliary apparatus and methods employed in determining the respiratory exchange and energy production of a subject in the chamber are explained in detail.

CONSTRUCTION OF THE RESPIRATION CHAMBER

The respiration chamber is approximately 1.96 meters long, 1.96 meters high, and 1.19 meters wide, the total volume of the empty chamber being close to 4,570 liters. On the side walls are hooks for clothing and shelves for books, food receptacles, and the like. The furniture consists of a chair and a table, and a cot is provided in experiments lasting a day or more. These may be folded into small bulk when not in use, to provide as much space as possible in which the subject may move about, if the nature of the experiment allows freedom of muscular movement. In experiments of several hours' duration, when the subject is to be very quiet, the ordinary chair and the cot are replaced by an adjustable reclining chair in which he may sit or recline at will, the change in position involving almost no effort. When the experiment involves the performance of muscular work, an ergometer of special construction for measuring the amount of muscular work done is included. There is a telephone for communication between the subject inside the chamber and the observer on the outside. Every provision is made for the convenience of the subject within the limits of the experimental conditions. (See Pl. XXXV, fig. 1.)

In one wall of the chamber, facing a window of the laboratory, there is an opening about 48 cm. wide by 54 cm. high, through which the subject enters and leaves the chamber (Pl. XXX). During an experiment this is closed with plate glass sealed in place, and thus serves as a window. On bright days this window will admit sufficient light for reading or writing, but further light is generally provided by a small electric lamp inside, which the subject may locate according to his desire. Near the center of one end of the chamber is a smaller opening through the walls, called the "food aperture," which is closed by a tube having a valve or trap on one end opening into the chamber, and another on the other end opening to the exterior. This comprises an air lock, through which articles such as food receptacles, books, etc., may be passed into or out of the chamber without any interchange of air between the interior and the exterior of the chamber other than that due to displacement by the articles placed in the

aperture. Several small openings in the walls provide for the passage of air pipes, water pipes, and wires for electric current (Pl. XXXII, fig. 1).

The walls, ceiling, and floor of the chamber are of 16-ounce copper, tinned on both sides. Large sheets of copper are used, so that there will be few joints in the walls. The sheets are joined with tightly locked seams heavily soldered, making them air-tight. When the soldering was completed, the tightness of the walls was tested by air pressure, the level of the column of water in a manometer connected with the chamber being observed at frequent intervals for several hours. It remained constant, due allowance being made for the effect of change of temperature or barometric pressure during the test.

The copper-walled chamber is attached to the inside of a framework of structural iron (Pl. XXXI, fig. 2). The sills and ceiling plates are angle iron with legs about 63 by 63 mm., and are bolted together at the corners. The studding for the side walls and the joists for the floor and the ceiling are of light-weight channel iron about 63 mm. wide, bolted to the plates with stiff angles or elbows, with the width of the channel at right angles to the length of the plates (Pl. XXXI, fig. 1). The chamber is attached to the framework by long, slender stove bolts passed through holes in the edge of the channels and screwed into brass nuts soldered to the outer surface of the copper. Between each channel and the copper attached to it is a strip of wood about 6 mm. thick and 3.5 cm. wide, to prevent actual metallic contact and to interfere with the transference of heat from the copper wall to its iron supporting structure. Between the copper floor and the floor joists is a layer of asbestos lumber about 9 mm. thick (shown in Pl. XXXI, fig. 1), to provide a solid support for the thin metal floor of the chamber.

To the outer edge of the iron structure is attached a surface of sheet zinc corresponding to the copper wall, ceiling, and floor of the chamber (Pl. XXXII, fig. 1). Sheet zinc about the same weight as that of the copper was used. Washers slipped under the heads of the bolts by which the copper wall is attached serve to bind the zinc to the iron. The chamber is thus provided with double metal walls separated by a dead-air space about 7 cm. across, the purpose of which is explained on page 331, in the description of the method of preventing the passage of heat through the walls of the chamber.

The framework of the chamber was made of structural iron, to secure rigidity and to provide a strong support for any apparatus that it might be found advantageous to employ in experiments in which muscular work would be performed. It entails, however, an undue amount of care in making the calorimetric measurements to avoid error that might result because of the heat capacity and thermal conductivity of the iron, as explained on page 338. Should opportunity to reconstruct the apparatus arise, the iron would be replaced by some material that would provide ample rigidity and strength of structure and have less thermal capacity and conductivity.

The chamber does not rest upon the floor of the laboratory, but is supported about 45 cm. above it by a structure of channel iron (Pl. XXXII, fig. 1), with upright pieces 10 cm. wide, which rest on floor plates and are bolted to the ceiling and between which are cross pieces 7.5 cm. wide, on the lower of which rests the chamber. To this structure is also attached the framework for supporting an outer covering of cork board, described on page 334. This covering is constructed so that it may be easily detached to provide ready access to any part of the zinc wall. The outer surface of the cork board is covered with a layer of museum board 6 mm. thick, painted white on the outside (Pl. XXX).

DETERMINATION OF RESPIRATORY EXCHANGE IN THE CHAMBER

The atmosphere of the empty chamber contains oxygen, nitrogen, water vapor, and carbon dioxid in proportions like those of ordinary air. When the subject enters the chamber, the proportions begin to change, with the consumption of oxygen and the elimination of water vapor and carbon dioxid. The removal of the water vapor and carbon dioxid from the air and the restoration of oxygen to it in such manner that the quantity of each may be accurately measured form the basis of the determination of the respiratory exchange in the chamber.

The respiratory products are constantly carried out of the chamber by a current of air that is kept in circulation through the system. The air leaves the chamber in a pipe which opens near the floor at one end, passes through purifying devices, and returns to the chamber in a pipe which opens near the ceiling at the other end. The purifying devices, called "absorbers," remove from the air passing through them the water vapor and carbon dioxid imparted to it by the subject. The increase in the weights of the absorbers in a given period shows the quantities of water vapor and carbon dioxid carried out of the chamber during the period. In addition to the data thus obtained, account must be taken of changes in the quantities of water vapor and carbon dioxid in the air of the chamber, as shown by analyses of samples of the air at the beginning and the end of the period, in determining the quantities produced in the chamber during the period (p. 310).

Oxygen is supplied to the chamber from a cylinder of the gas under pressure, and the loss in weight of the cylinder shows the quantity admitted during the period. To determine from data thus obtained the quantity of oxygen consumed by the subject, allowance must be made for changes in the quantity of oxygen in the air of the chamber.

AIR-TENSION EQUALIZER

The volume of air in the chamber varies constantly with the admission of oxygen and the removal of water vapor and carbon dioxid, and also with changes in the temperature of the air in the chamber and in the barometric pressure of that outside. This might result in undesirable

variations in the pressure of the air in the chamber unless provision were made for corresponding fluctuations in the capacity of the system. This is accomplished by attaching a flexible diaphragm of thin rubber or a sensitive spirometer to a small tube opening into the chamber, which serves as a tension equalizer, keeping the air of the chamber always at the barometric pressure of that of the laboratory (Pl. XXX).

AIR-PURIFYING SYSTEM

The circulation of air is maintained by a rotary air pump, which has a capacity of close to one-fourth of a liter per revolution and is driven at a rate of about 250 revolutions per minute, so that the air is forced through the purifying system at a rate of 60 to 70 liters per minute. An electric motor of one-eighth horsepower is sufficient to run the pump and to move the air through the absorbers (Pl. XXXII, fig. 2).

All piping in the air-circulating system is brass pipe of the so-called half-inch size, which has an internal diameter of 15 mm. The apertures of the air passages in the purifying devices are also of this size. This has been found sufficient to conduct the air at the desired rate without undue resistance, the pressure in the section of pipe between the compressor and the first water absorber, where it is higher than in any other part of the system, being less than 40 mm. of mercury.

The motor, the rotary air pump, and the absorbers for water vapor and carbon dioxide are assembled on a suitable-sized stand or table, with three shelves, called the "absorber table" (Pl. XXXIV, fig. 1). The motor and pump are on the lower shelf, and on the middle shelf are the purifying devices in a series or train; first, the absorbers for water vapor, and next, the absorbers for carbon dioxide. The air pipe from the respiration chamber passes to the pump and then to the inlet end of the absorber train. From the outlet end of the train the air pipe returns to the chamber, the ingoing and outgoing pipe passing through the walls in two apertures close together. Inside the walls the pipes extend to opposite ends of the chamber, the end of the ingoing pipe being near the top of the chamber, and that of the outgoing pipe near the bottom.

Two absorber trains are set up in parallel and are used in alternate periods, the air pipe at each end of the trains being branched for this purpose. There is a valve in the piping at each end of each train, and the change from one train to the other involves merely closing the valves for one train and opening those for the other. When ordinary wheel valves are used, as shown in the illustration, the motor is stopped for the few seconds necessary to make the change; but the valves at each end of the purifying system may be replaced by a suitable 3-way cock or air trap at the point where the air line branches at each end of the train, and the two cocks may be actuated by the same shaft, so that the air current can be shunted from one train to the other with a single motion from either end of the absorber table and while the air pump is

still running. By actuating the shaft electrically the change can be made by the observer at a distance, or a clock can be used to close the electric circuit at any given time and thus make the change automatically.

While the air is passing through one train the other is disconnected, the absorbers weighed, the absorbent renewed if necessary, and the train again connected in position. The absorbers are joined together by couplings which are attached to the inlet and outlet tubes by stout, flexible rubber tubing. Rubber washers between the halves of each coupling make a tight joint. A similar coupling connects each end of the train with the air pipe. When the whole train is in position it is tested for tightness, with the air in the system at a pressure of about 1 meter of water, which is considerably more than the highest pressure in any part of the train in service.

REMOVING WATER VAPOR FROM THE AIR

In the purifying system the air passes first through sulphuric acid, which removes all water vapor from it. The acid container, which is in effect a modified gas-washing bottle of moderately large capacity (Pl. XXXIII, fig. 1), was devised in connection with these investigations. A strong glass bottle about $2\frac{1}{2}$ liters in capacity (about 24 cm. in height and 12 cm. in diameter), with a wide mouth, is fitted with a special ground-glass stopper, in the top of which are sealed an entrance and an exit tube, each 15 mm. in internal diameter. The entrance tube, which is in the middle of the stopper, extends to very near the bottom of the bottle, and terminates in a bulb about 4.5 cm. in diameter, which has several holes about 4 mm. in diameter in the sides and bottom, the total area of the holes being about equal to that of the cross section of the tube. Surrounding the bulb is a bell of about 7.5 cm. diameter, attached to the tube at a point a little above that at which the bulb is attached. The bell is completely open at the bottom, and has a row of holes about 7 mm. in diameter around the side at a level just above the top of the bulb.

When charged, the bottle is filled with acid to a level a little above the row of holes in the bell, about 750 c. c. of acid being sufficient for this purpose. The air escaping through the holes in the bulb and in the bell is broken into bubbles, which in passing through the acid are deprived of moisture. The passage of the air through the acid keeps it vigorously stirred, acid coming up through the bottom of the bell to replace that forced out through the holes at the sides. To prevent globules of acid from being spattered or carried by the air into the exit tube, the bottom of the stopper, which is about 6 cm. below the top, is nearly closed, an annular space about 8 mm. across being left around the tube that projects to the bottom of the bottle to provide for the exit of air. Into the space thus formed in the interior of the stopper are placed lumps of

pumice stone, which effectually prevent visible particles of acid from being splattered into the exit tube or carried into it by the air current.

During several years' use these bottles have proved to be very satisfactory. Before they were used in experiments a large number of tests of their efficiency were made, in which air was passed at various rates up to 80 liters per minute through three of the bottles in series, the first one containing water, in which the air became very moist, and the other two charged with acid. It was found that the moist air leaving the first bottle could be passed through the acid in the second bottle until it was diluted to nearly twice its bulk before the third bottle increased appreciably in weight. No gain in weight was ever observed in a third acid bottle included in the series in some of the tests. In many of these tests the water vapor in the air leaving the water bottle was very nearly saturated at the temperature of the laboratory. These conditions imposed as severe a test on the capacity of the device to remove all moisture from the air flowing through it as any that would occur in respiration experiments.

In practice, two bottles are used in series and the first one is recharged when the acid in it has become diluted to a volume indicated by a mark on the bottle, in which case 750 c. c. of acid have usually absorbed 500 to 600 c. c. of water. Each bottle with its charge of acid weighs not far from 2,600 gm. The two acid bottles will stand side by side on the pan of the large sensitive balance, and are weighed together to an accuracy of 0.1 gm. The increase in the weight of these two absorbers in a given period shows how much water vapor has been carried out of the chamber during the period.

REMOVING CARBON DIOXID FROM THE AIR

The air from the acid bottles passes next through bottles containing soda lime (a mixture of caustic soda and quicklime), which deprives it of carbon dioxide. The soda-lime container that has been in use for several years consists of an ordinary wide-mouth bottle about 25 cm. in height and 13 cm. in diameter. The mouth of the bottle is closed with a No. 12 rubber stopper, through which pass an inlet tube and an outlet tube of brass pipe, with a bore of 15 mm. The inlet tube extends nearly to the bottom of the bottle. The lower opening of this tube is protected with brass wire gauze to prevent particles of soda lime from entering it. The outlet tube extends outward from the under side of the stopper. When the stopper is tightly sealed and bound in place, soda lime in particles about the size of a dried pea or smaller is introduced through the outlet tube until the bottle is filled quite near to the top. Each bottle when thus charged contains a little over 2 kgm. of soda lime and weighs about 4 kgm.

Two of these bottles are used in series, and each one is kept in use until the appearance of the soda lime indicates that it is no longer effi-

cient enough for further use, which is shown by its change in color. The fresh, somewhat moist soda lime is a dingy white, but in use it becomes much lighter and clearer, owing to both the absorption of carbon dioxid and the loss of moisture, which is taken from the soda lime by the dry air. The bottle may be recharged whenever all of the visible surface of soda lime has thus changed, though if the whitened material has not become compacted into a hard mass which will prevent air from passing through it the efficiency of the soda lime may be restored by passing air containing water vapor through the bottle until the dry material has absorbed about as much moisture as it contained originally, as may be judged from the darkening of the color. In this manner a given charge may be used at least twice. In either case, if the bottle is opened, any soda lime not compacted but still remaining granular may be used again, especially if it is mixed with a large proportion of fresh material. In an ordinary rest experiment in which carbon dioxid is removed from the air current at a rate of 25 to 30 gm. an hour, the material in one of these bottles will absorb at least 150 to 200 gm. of carbon dioxid before all the soda lime has whitened.

These bottles are quite satisfactory in many respects, but in using them great care is necessary to avoid leakage of air between the stopper and the neck of the bottle, or between the stopper and the tubes passing through it, especially after the bottle has been in use a short time. When these joints are made, they are thoroughly painted with shellac, but since the stopper is quite flexible there is possibility of breaking the coating in using the bottle. Some of these chances for leakage will be eliminated by a special cover designed to be clamped to the top of the bottle, into which the inlet and outlet tubes are soldered.

The soda lime is used moist rather than dry because it is more efficient in that condition. In passing through this moist material the dry air from the water-vapor absorber takes moisture from it. The air from the carbon-dioxid absorber is therefore passed through another bottle of sulphuric acid, to catch the moisture given off by the soda lime. This bottle is weighed with the two soda-lime bottles to find the amount of carbon dioxid removed from the air current coming from the respiration chamber, the three bottles standing together on the pan of the large balance being weighed as a unit. Their total weight, which is less than 12 kgm., is ascertained accurately to 0.1 gm.

TRAP FOR ATOMIZED SULPHURIC ACID

Though the pumice in the stopper of the sulphuric-acid bottle effectively arrests visible particles spattered up by the vigorous agitation of the acid or blown up in the air current, acid in some condition, apparently resembling vaporous exhalation, escapes in the air leaving the bottle. The amount of acid that leaves the absorber is so small that even after the air

has been passing for several hours the loss has no effect on the weight of the absorber within the limits to which the weight is determined; yet if the acid carried in this manner from the bottle mentioned in the preceding paragraph is allowed to escape into the air of the chamber, it has a noticeable effect upon the respiration of the subject in a few minutes. To avoid this effect, the air from the absorber passes through a trap which removes the acid spray before it enters the pipe for air returning to the chamber. For several years the trap consisted of sodium carbonate between two layers of cotton wool inclosed in a metal cylinder about 15 cm. long and about twice the diameter of the air pipe. Later, a piece of heavy glass tubing was substituted for the metal cylinder (Pl. XXXIV, fig. 1), and it was observed that the air was freed from acid apparently by mechanical filtering rather than by chemical action between the acid and the carbonate. The first layer of cotton arrested all the acid that reached the trap during several months' use, and the carbonate appeared to be unnecessary. In accordance with this supposition, the cotton and carbonate in the trap were replaced by pumice stone in pieces very much smaller than those in the stopper of the absorber, and this has prevented the passage of the acid spray into the pipe for ingoing air.

SUPPLYING OXYGEN TO THE AIR

Oxygen to replace that used by the subject is admitted directly to the chamber through a copper pipe of a bore of about 5 mm. passing through an opening in one wall. The supply of oxygen is contained under pressure in a steel cylinder, the outlet of which is closed with a pressure-regulating valve by which the rate of admission of oxygen is governed. No attempt is made to keep any definite proportion of oxygen in the air. The regulator valve is usually set to admit oxygen at a rate that will keep the volume of gas in the chamber fairly constant, as indicated by the rubber diaphragm or the spirometer serving as an air-tension equalizer for the chamber. The valve may be opened or closed by hand as regulation of the volume is necessary; or by causing the diaphragm or spirometer when nearly full to open and when nearly empty to close an electric circuit, an auxiliary valve may be operated so that the admission of oxygen is automatically regulated to keep the total volume of air in the chamber within the desired limits. A simple auxiliary valve consists of a pinch-cock actuated by an electromagnet so as to compress or release the rubber tubing connecting the outlet of the regulating valve with the end of the pipe taking oxygen to the chamber.

The steel cylinder containing the oxygen is suspended from one arm of a large sensitive balance, and from the other arm is suspended a similar cylinder, empty, to serve as a counterpoise (Pl. XXXIV, fig. 1). The loss in weight of the charged cylinder in a given period shows the amount of gas admitted to the chamber during the period. Though each cylinder

weighs nearly 60 kgm., the loss in weight is ascertained to an accuracy of 0.1 gm.—that is, the volume of gas supplied, which may reach 80 liters or more per hour, may be determined within 100 c. c.

This method of determining the quantity of gas admitted to the chamber is very precise, but it involves time and effort that could be saved by the use of a gas meter if the mere reading of the dial of the meter would show the quantity with equal precision. In a number of experiments the gas from the weighed cylinder was passed through a calibrated test meter before it entered the chamber, to determine whether the volume of gas admitted could be ascertained in this manner with sufficient accuracy. It was found that when the gas was admitted at a fairly uniform rate throughout the period, the volume as determined from the meter reading would agree quite closely with that computed from the loss in weight of the cylinder; but when it was necessary at times to admit gas rapidly, the agreement was not so close, a correction being necessary for increase of pressure in the meter. The time and labor involved in reading, recording, and correcting for increased pressure in the meter are at least as much as those of weighing the cylinder.

In most of the investigations with this respiration calorimeter the gas contained in the cylinder, and consequently that admitted to the chamber, was about 97 per cent oxygen. It was derived from liquid air and was virtually free from carbon dioxide and water, but contained a small proportion (about 0.3 per cent) of nitrogen and an appreciable proportion (about 2.7 per cent) of argon, for which allowance must be made in computing from the loss in weight of the cylinder the quantity of oxygen admitted to the chamber. In making the correction it is sufficiently accurate to consider the impurity as all argon. It is possible, however, to obtain oxygen that is so nearly free from other gases that the error involved in disregarding them is inconsiderable.

DETERMINATIONS OF THE AMOUNTS OF RESIDUAL GASES

As has been stated (p. 304), to determine the amount of oxygen consumed and of carbon dioxide and water vapor produced by the subject in the chamber during a given period, allowance must be made for any changes that have occurred in the composition of the air of the chamber—that is, in the quantities of different gases residual in the chamber. These are ascertained from analyses of samples taken at the beginning and the end of the period. Because of convenience, the samples are taken, not directly from the air of the chamber but from that passing through the air pipes outside of the chamber. It is assumed that the air in the outgoing pipe has the same composition as that in the respiration chamber. Though the composition of the latter is constantly changing, an electric fan keeps the total mass of air in the chamber energetically stirred to prevent stratification and to mix the varying component gases as thoroughly as possible. It seems probable, there-

fore, that the composition of the air in the outgoing pipe fluctuates quite uniformly with that of the total air in the chamber.

ANALYSIS OF SAMPLE FOR WATER VAPOR AND CARBON DIOXID

For the determination of the amounts of moisture and carbon dioxide residual in the chamber at the end of each period, a portion of the air coming from the chamber at that time is shunted from the main current through a petcock in the air pipe at a point between the rotary pump and the first sulphuric-acid bottle, and is passed first through a small purifying system and then through an accurate gas meter, which rests on the top shelf of the table for the large absorbers, as seen in Plate XXXVI, figure 2. The air leaving the meter is passed through sulphuric acid to remove the water vapor taken up by it in passing through the meter, and is then returned to the main current flowing from the large absorbers to the chamber. The water-vapor absorbers of the small train are specially devised, somewhat resembling those of the large train, but of such size that they may be weighed on an analytical balance (Pl. XXXIII, fig. 2). A 4-inch U tube with side outlets and well-ground glass stoppers makes a serviceable soda-lime container. A train consisting of one acid bottle, one U tube, and another acid bottle very efficiently removes all water vapor and carbon dioxide from the air passing through it at a rate of about 3 liters per minute.

The small absorbers are weighed on an analytical balance to an accuracy of 0.1 mgm., each unit, when charged, weighing less than 100 gms. The increase in the weights of the units shows the quantities of water vapor and carbon dioxide in a given volume of the air. Usually 10 or 20 liters of air, as indicated by the meter, are passed through the train, the actual volume being ascertained by correcting the meter reading, when necessary, for the calibration of the meter and for the temperature and barometric pressure of the air passing through it.

ANALYSIS OF SAMPLE FOR OXYGEN

For the determination of the proportion of oxygen in the residual air a small sample, about $\frac{1}{2}$ liter, is taken from the returning air in the pipe between the large purifying system and the respiration chamber, where it is free from water vapor and carbon dioxide. In Plate XXXVI, figure 2, a rubber bag for holding the sample is seen hanging from an outlet in the air pipe at the end of the absorber table. A modified Haldane burette is used in the determination, the oxygen being absorbed by a potassium-pyrogallate solution in a Hempel pipette.

COMPUTATION OF VOLUMES OF GASES PRESENT

The actual determination of the proportion of oxygen in the air is not necessary at the end of each period. The volume of oxygen present in the air of the chamber may be computed by subtracting from the actual

volume of total air present the sum of the volumes of carbon dioxid and water vapor present, as shown by analyses of the residual air, and the volume of nitrogen, including that present at the beginning of the period and that added with the oxygen admitted during the period, due allowance being made in the latter for any impurity.

To compute the total quantities of carbon dioxid and water vapor in the air of the chamber, the volumes corresponding to the weights of the gases removed by the small absorber system from the air sample measured by the meter are multiplied by a factor representing the ratio between the volume of the sample and the total volume of air in the chamber when both are reduced to standard conditions of temperature (0°C.) and of pressure (760 mm. of mercury). The necessity for accuracy in the analysis of the sample is shown by the fact that under usual experimental conditions there are more than 4,000 liters of air in the chamber; hence, any error in the determination of the quantities of water vapor and carbon dioxid in a 10-liter sample is multiplied over 400 times.

The actual volume of air in the chamber under standard conditions depends upon the capacity of the chamber and the barometric pressure and temperature of the air in it. These factors must be accurately determined, since a difference of 1 mm. in the pressure means a difference of over 5 liters in the computation of the actual volume of gas, while a difference of 1 degree in the temperature means a difference of about 15 liters in the total volume. An error in these determinations has some effect upon the computation of the quantities of residual gases, though the effect of any error likely to occur upon the quantity of water vapor would be quite insignificant, as there are seldom more than 90 liters present, and commonly less. The effect on the computation of carbon dioxid would be somewhat larger, as there might be in some circumstances 100 liters or more in the air; but under ordinary conditions the quantity is decidedly less, and the error would be relatively unimportant. The effect would be greatest upon the computation of the quantity of oxygen, as under normal conditions there could be as much as 850 liters present.

MEASUREMENT OF CAPACITY OF THE CHAMBER

The capacity of the chamber is known very accurately. It may be computed from the dimensions of the chamber, and it may be directly ascertained by determining the proportion of oxygen in the well-stirred air of the sealed chamber before and after the admission of a known volume of the gas.

MEASUREMENT OF BAROMETRIC PRESSURE OF THE AIR

The barometric pressure of the air of the chamber, which, because of the air-tension equalizer mentioned on page 304, fluctuates the same as

that of the laboratory, is determined by means of an accurate barometer mounted on the walls of the laboratory. The height of the mercury column in the barometer tube may be read by a vernier to 0.01 mm. The barometer has been standardized by the Weather Bureau.

MEASUREMENT OF TEMPERATURE OF THE AIR

The temperature of the total mass of air in the chamber is not so easily determined as its pressure. Even when the walls of the chamber are at uniform temperature and no heat is generated in it, the temperature of the air may not be uniform in all parts of the space. When heat is being generated in the chamber and is being absorbed and removed as fast as it is generated, so as to maintain constancy in what is assumed to be the average temperature, there is a considerable difference between the temperature and the consequent density of the air near the source of heat and that of air near the heat absorber. It seems reasonable to suppose, however, that with the tendency of warm air to rise and of cold air to fall, and particularly with the vigorous agitation of the air of the chamber by the electric fan, the warmer and colder volumes of air will be very rapidly mixed, and more or less complete uniformity of temperature quickly established throughout the whole mass of air.

The temperature of the air of the chamber is measured by means of an electric-resistance thermometer. The method of measurement employed is based upon the fact that the resistance of a wire of pure metal to an electric current changes definitely with a change in its temperature and also that the resistance of the wire, and particularly its change in resistance, whether large or small, due to corresponding changes in temperature, may be measured with extreme accuracy by means of a suitable Wheatstone bridge. The device used in the respiration calorimeter comprises specially mounted bare nickel resistance wire in the chamber, connected with a special Wheatstone bridge, called the "temperature indicator," on the observer's table (Pl. XXXVI, fig. 1).

The nickel wire, the resistance of which varies with changes in the temperature of the air of the chamber, is in six coils of equal resistance, each of which is mounted in a rectangular wooden frame about 10 by 13 cm. that is suspended in the air about 4 cm. from the wall of the chamber, on supports attached to the wall. The wire is stretched across the space in the frame between two slender wooden rods about 5 cm. apart, with successive strands of the coil about 5 mm. apart. Since very little of the wire is in contact with the support, it is but little, if at all, affected by the temperature of the frame, the object of the construction being to eliminate lag in the action of the thermometer. The exposed wire very rapidly acquires the temperature of the air of the chamber, and hence responds instantly to any changes in it. The six coils are distributed on the walls and ceiling in different vertical and horizontal positions, to

integrate different temperatures if there are differences, and as the air is very thoroughly stirred by the electric fan previously mentioned, it is probable that the resistance thermometer shows the average temperature of the air of the chamber. In the interior view in Plate XXXV, figure 1, two of the frames are plainly shown with a wide-mesh wire screen before the resistance wire to protect it against contact with any object that would cause a short circuit between two parts of the wire, as well as against injury.

The six coils are connected in series by well-insulated No. 16 copper wire, and similar wire leads from the terminals of the series, through a rubber stopper in a small opening in one wall of the chamber, to a special switch on the observer's table, by which they may be connected in one arm of the Wheatstone bridge. The purpose of the switch is to provide means for using with these coils the same bridge that is used with other coils for measuring the temperature of the walls of the chamber and that of the body of the subject, as explained later in this paper. This switch must be designed to avoid the error that would result from introducing appreciable resistance of the switch contacts into the bridge circuits. The connections between the bridge and the resistance coils include a compensating lead to eliminate from the measurement of the resistance of the coils the effect of both the resistance of the leads and any change in their resistance due to change of temperature. The contact that is moved along the slide wire of the bridge, to restore balance when the resistance of the thermometer coils has changed, is in series with the battery, so that contact resistance introduces no error in the measurement.

The six coils have a total resistance of about 20 ohms at 20° C. Since the resistance of nickel wire varies approximately 0.4 per cent per degree at the usual temperatures of the experiments, their total change in resistance would be close to 0.08 ohm for a change of 1° in the temperature of the air of the chamber. The resistance of the slide wire of the Wheatstone bridge will balance the bridge circuit for the change of resistance in the coils that would result from a change of 5° in the temperature. By means of several coils of manganin wire, which may be connected in series with the slide wire, the total range of the bridge may be extended, but under usual experimental conditions the temperature of the air is allowed to change as little as possible. Whether the change is large or small, it must be measured accurately. A change of resistance in the thermometer coils resulting from a change of 0.01° in the temperature of the air will upset the balance of the bridge sufficiently to cause a deflection of the sensitive reflecting D'Arsonval galvanometer that indicates when the bridge is balanced. The balancing point of contact may be moved along the wire a distance sufficiently small to restore the balance, and the scale of the slide wire will indicate the distance.

OBSERVER'S TABLE

The Wheatstone bridge described above and the telephone mentioned on page 302 are located on the table beside the chamber (Pl. XXXVI, fig. 1) at which the observer sits while controlling the apparatus. The same bridge is employed in the determination of other temperatures, as described beyond. Other devices on the table serve to indicate and regulate temperature conditions inside and outside the chamber, as explained in detail in the sections which follow.

DETERMINATION OF THE QUANTITY OF HEAT PRODUCED IN THE CHAMBER

Energy expended by the human body for any purpose, such as the performance of muscular work, the maintenance of body temperature, or whatever, results in the production of heat, which is eventually dissipated from the body; hence, the measurement of the quantity of heat dissipated by the body under given conditions affords data for the determination of the quantity of energy expended. Heat escapes from the body in two ways: As latent heat of water vaporized from the lungs and skin and as sensible heat, by conduction, convection, and radiation from the surface of the body to the air and to objects in the chamber. Both latent heat and sensible heat are carried out of the chamber and measured.

MEASUREMENT OF LATENT HEAT

The water vaporized by the lungs and skin leaves the chamber in the outgoing air, unless it is precipitated by contact with some object in the chamber whose temperature is below the dew point for the conditions prevailing, but the temperature of the air and of objects in the chamber is controlled so that precipitation is not likely to occur. The quantity of heat leaving the chamber as latent heat of water vapor in any given period is determined by multiplying the weight of the water vapor absorbed from the outgoing air during the period by the factor 0.586, which according to determinations made by Smith (18), represents the number of Calories of heat required to vaporize a gram of water at 20° C. All measurements of heat with the calorimeter are expressed in terms of Calories at 20° C., 1 Calorie being taken as the amount of heat required to raise the temperature of 1 kgm. of water 1° C.—i. e., from 19.5° to 20.5°, the specific heat of water being taken as unity at 20° C. The determinations by Smith were made in accordance with the conclusion by Barnes (7) that the mean small calorie is equivalent to 4.1877 international joules. Dickinson, Harper, and Osborne (10), in work on the latent heat of fusion of ice, assumed 4.187 international joules equal to 1 small calorie at 15°, in which case 4.183 joules would be equivalent to 1 small calorie at 20° C. The latter value is used in these investigations (p. 342), but the difference between this and the value by Barnes has no significant effect upon the factor for latent heat here employed.

MEASUREMENT OF SENSIBLE HEAT

The energy eliminated from the body as sensible heat, which is much greater in amount than that latent in water vaporized from the body, is practically all carried out in a current of water which circulates in the chamber through a device called the "heat absorber," though a small quantity of it may become latent in water vaporized from objects in the chamber, in which case it may leave the chamber as latent heat of water vapor in the outgoing air. If the weight of the water that flows through the absorber during a given period, as stated in kilograms, is multiplied by the difference between the temperature of the water as it enters and that as it leaves the absorber, as measured in degrees centigrade, the product will show the quantity of heat removed as expressed in Calories, at the mean temperature of the water flowing in the absorber. These are converted into Calories at 20° by making due allowance for the specific heat of water at the mean temperature of the flow as compared with that at 20° (4, p. 56; 19, p. 229).

The rate at which heat is removed from the chamber is regulated to prevent fluctuations in the temperature of the air of the chamber, which falls when the rate is too fast and rises when it is too slow. To avoid chance for error in the determination of the volume of air in the chamber, which depends upon the accuracy of the measurement of its temperature (p. 313), and to some extent also for the comfort of the subject, it is desirable to keep the temperature of the air as constant as possible. The temperature to be maintained depends upon the nature of the experiment, but it is commonly not far from 20° C. Whatever the requirement may be, by proper control of the temperature at which the water enters the heat absorber, and of the rate at which it passes through the absorber, the removal of heat from the chamber may be made to accord with its production within it to such an extent that the temperature of the air of the chamber may be kept constant within narrow limits. The most convenient practice is to maintain a constant rate of flow and to vary the temperature of the water entering the heat absorber according to the amount of heat to be absorbed.

HEAT ABSORBER

The heat absorber, which is suspended near the ceiling of the chamber, about 10 cm. from the sides, consists of brass pipe of 7 mm. internal diameter (so-called $\frac{1}{8}$ -inch pipe), along which disks of sheet copper 5 cm. in diameter are soldered 3 mm. apart to increase the area of the heat-absorbing surface. The total length of pipe in the absorber is not far from 11 meters, and there are more than 2,500 disks on it, so that several square meters of surface are exposed to the air of the chamber. Though the total quantity of water in the absorber is not over 400 c. c., it is possible, by control of the temperature and rate of flow of the water, to vary the rate of removal of heat from the chamber within wide limits.

The coil passes once around the chamber and back again, the two pipes lying not quite 5 cm. apart, with the disks on one slightly overlapping those on the other. The purpose of this arrangement is to establish as much uniformity as possible in the absorption of heat from the air enveloping the absorber. Incidentally this would result in corresponding uniformity in the density of the air affected by the absorber.

REGULATING AND MEASURING THE WATER FLOW

Water for the heat absorber is drawn from a small tank several feet above the ceiling of the chamber, which is filled by water flowing from the city main. An overflow pipe in the tank keeps the water supply at a constant level; and since the level at which the water leaves the absorber is also fixed, the pressure in the system is constant. Under favorable conditions the rate of flow through the absorber is quite regular. At times, however, in cold weather, when a considerable amount of air is dissolved in the water, some of the air that is liberated when the temperature of the water is raised gradually accumulates in the absorber and reduces the rate of flow in an irregular manner. Under these conditions the faster the rate, the more constant it is. For this reason a specially devised rate valve is of only limited service in regulation of the rate of flow, though it has some advantages over the common stopcock.

The water leaving the heat absorber flows into a copper cylinder holding about 3 liters and through a stopcock in the bottom of this into a tank holding about 100 liters. This tank will catch all the water that would leave the heat absorber during a period of at least three hours, in experiments in which the dissipation of heat in the chamber is about 100 Calories per hour, a rate of flow of 350 to 450 c. c. of water per minute, with the temperature of the ingoing water about 16°, having been found quite satisfactory in such circumstances. The large tank rests upon a sensitive platform balance (Pl. XXX) by which the weight of the water is determined to 0.01 kgm. The small cylinder catches the water that flows while the tank is being weighed and emptied.

REGULATING THE TEMPERATURE OF WATER ENTERING THE HEAT ABSORBER

The temperature of the water entering the heat absorber is so completely under control that it may be kept indefinitely at any desired point within narrow limits, or may be changed rapidly, if necessary, from one point to another. To accomplish this, the water is first cooled to a temperature below that at which it will be used and then brought to the required temperature by electric heating. In these circumstances, when any change in temperature is desired, it is necessary to vary only the heating. The chilled water passes into a device called the preheater, which does the greater part of the heating necessary to warm the water to the desired temperature. The heating effect of this device is adjusted by hand. From the preheater the water flows into the bottom of a large

bottle filled with pieces of pumice as large as will pass through the narrow neck. In this reservoir the water is mixed so that any change in the temperature of that entering the bottle, due, for instance, to fluctuations in the voltage of the current in the preheater, will be dissipated through the mass to such extent that there will be no rapid fluctuations in the temperature of the water leaving the bottle. From this reservoir the water enters the final heater which completes the heating necessary to bring the water to the desired temperature. This device functions automatically and varies the amount of heating it does to accord with the fluctuations in temperature of the water coming from the mixing bottle. From the final heater the water flows into a smaller mixing bottle, from which it passes to the heat absorber.

WATER COOLER

To cool it, the water from the pressure tank is passed through a coil of pipe submerged in cold water, in a tank nearly 1 meter in length by 30 cm. in width and depth and containing 80 to 90 liters of water. The coil consists of nearly 6 meters of iron pipe, of 15 mm. bore, in six parallel rows running from end to end near the bottom of the tank. The water in the tank is chilled by cold brine flowing through a second coil, immersed in the water above the former coil. A small ethyl-chlorid refrigerating machine keeps the temperature of the circulating brine quite uniform. In this manner the temperature of the water leaving the cooling coil is readily kept below that at which it may be needed at any time during an experiment, and fairly uniform, but it can not be regulated by cooling alone as closely as needed for use in the heat absorber.

WATER HEATER ADJUSTED BY HAND

The preheater consists of several coils of electric-resistance wire of different sizes wound upon a thin-walled brass tube about 16 mm. in diameter, from which they are insulated with mica. Outside of this is a similar tube about 26 mm. in diameter, and the annular space between the two and surrounding the resistance coils is filled with sand, so that the heat generated by the electric current in the resistance wire is transmitted rapidly to both tubes. This heater is mounted inside a brass tube 37 mm. in diameter, in such manner that the chilled water, entering the large brass tube, flows in one direction along the outside of the heater and returns along the inside, absorbing all the heat generated in it. By means of plug switches on the base supporting the heater various combinations of the coils may be put into service, as desired, to vary the heating. By the use of this device the temperature of water flowing at a rate of about 1 liter a minute may be increased nearly 10 degrees, if desired, in increments of about 0.25 of a degree.

There are seven resistance coils in the heater, of which four have a resistance of about 340 ohms each. There would be a little less than 0.65 ampere of current flowing in such a coil at 220 volts, which would give approximately 140 watts. To raise 1 degree the temperature of water flowing at the rate of 1 liter per minute requires approximately 70 watts; hence, each of these four coils would increase the temperature about 2 degrees. The resistances of the three other coils are, respectively, about 680, 1,360, and 2,720 ohms, and their output, respectively, about 70, 35, and 18 watts, with corresponding heating effects sufficient to raise the temperature of the water about 1, 0.5, and 0.25 degree.

If these coils were all wound in one tube, the heater would be inconveniently long. Two similar tubes, each 30 mm. long, are used, with the five coils of smaller resistance in one and the two coils of larger resistance in the other. The cold water flows first through the former and then through the latter. The two tubes mounted side by side on the same base may be seen in Plate XXXVI, figure 2, on a board attached to the side of the calorimeter.

WATER HEATER OPERATED AUTOMATICALLY

The final regulation of the temperature of the water for the heat absorber is done in a short tube inclosing a water channel, called the "final heater," which is shown in Plate XXXVI, figure 2, beside the preheater, on the board attached to the side of the calorimeter. In the upper end of the channel is an electric resistance thermometer coil that is connected with an indicator on which may be set the temperature at which it is desired to keep the water entering the heat absorber. In the lower end of the channel is an electric heating coil, in series with which is a rheostat for varying the current in the coil. The slider of the rheostat is adjusted by a screw shaft that is driven by a small electric motor. The water passing through the channel flows directly from the heater to the thermometer. If the temperature of the water flowing over the thermometer differs as much as 0.05 degree from that set on the indicator, the armature of the small motor turns in one direction or the other, depending on whether the water is too cold or too warm, and adjusts the rheostat until the current in the coil is just enough to heat the water to the desired temperature.

The water tube in this device, which is 28 cm. long, has a narrow channel, the cross section being 12 mm. in length and 4 mm. in width and having round ends. It was made by flattening thin-walled copper tubing of an external diameter of 1 cm. At each end the tubing is left circular in cross section and is soldered into a short nipple, which is screwed into one end of a special brass fitting with side outlets. Thin-walled brass tubing 2.5 cm. in external diameter, extending from one nipple to the other, forms a case around the channel, protecting it from

mechanical strain and surrounding it by a small dead-air space which serves to some extent as a heat insulator, protecting it from changes in temperature of the laboratory air. The side openings in the fittings provide an inlet and an outlet for the water.

The electric heater, which is in the lower end of the channel, consists of platinum wire, of 55 ohms' resistance, in a flat coil about 10 cm. long and 9 mm. wide, inclosed in a flat case of thin metal which, with the coil inside, is 10 mm. wide and 2 mm. thick. At one end this flat part of the case tapers into a tube about 3.5 cm. in length and 6 mm. in diameter, in which are the wires carrying electric current to the coil. This heater is inserted in the water channel, through the open end of the fitting, to the depth at which the whole of the resistance wire will be immersed in the water current, and a packing device in the end of the fitting is tightened around the neck of the case to hold the heater in place. In the channel the heater is surrounded by a space 1 mm. across, through which the water flows. Heat generated in the coil is imparted instantly to the water which surrounds the heater in such a thin layer that the temperature of the whole mass of water is very quickly affected.

The electric current flowing in the heating coil is determined by ballast resistance in series with the coil, of which 125 ohms are fixed and 550 ohms variable. For the former an ordinary resistance unit is satisfactory, and for the latter a rheostat of oxidized constantan wire of graduated cross section wound on an insulated light-steel tube has given excellent service. The sliding contact on the rheostat may be moved by hand or by means of a screw shaft (Pl. XXXV, fig. 2). When the total resistance of both the rheostat and the resistance unit is in series with the coil, a current of approximately 0.3 ampere will flow in the coil, the heating effect of which is sufficient to increase by a little less than 0.1 degree the temperature of water flowing through the heater at the rate of 1 liter per minute. When the whole of the rheostat is out of the circuit and only the resistance unit is in series with the coil, the current will be approximately 1.2 amperes, with a heating effect sufficient to increase by a little over 1 degree the temperature of 1 liter of water per minute.

Between these limits the heating effect may be varied in large or small steps, according to the distance the sliding contact is moved along the turns of wire in the rheostat. If the position of the slider is adjusted by hand, any portion of the rheostat, from the total resistance to that of a single turn of the wire, may be instantly put into or out of the circuit. That the temperature of the water may be automatically regulated, however, the position of the slider is adjusted by a screw shaft. A small pulley on the end of the shaft is belted to another pulley on the armature shaft of a small electric motor, that may be caused to run in one direction or the other and for a longer or shorter period, depending upon whether the amount of resistance in the circuit must be increased or decreased and how much. The field coils of the motor are differentially

wound, and the direction in which the armature of the motor will rotate depends upon the windings by which the fields are excited. With current flowing in both pairs of field coils alike the armature will not turn in either direction; but if one pair of coils is shunted, the effect of the other pair predominates, and the armature will rotate. The direction of rotation depends upon the closing of one or the other of two contacts in the circuit of the field windings, thereby shunting one or the other pair of field coils, and the duration of rotation depends upon the length of time the contact is closed.

The contacts are closed by keys which are depressed by cams on a light cam shaft driven by a small motor. The cams rotate continuously, and when a circuit is to be closed an idler swings between one of the cams and the key to be depressed. There are three cams for each key, differing in respect to the time each one presses on the idler, and the duration of contact depends upon which of these cams is engaged. Each idler is mounted on a lever which carries it into position between the cam and the key, the lever being actuated by a pin on the rotating shaft. A galvanometer needle decides which lever is to swing and to which of the three cams it is to be carried. The galvanometer, though incorporated in the device for making contacts, is connected with the indicator on which is set the temperature at which the water is to be kept. The direction and amplitude of deflection of the needle depend upon whether and to what extent the temperature of the water is above or below that set on the indicator. The galvanometer thus governs the direction and extent of motion of the slider on the rheostat which regulates the current in the heating coil in the water channel. The period of the galvanometer is less than 3 seconds, and the cam shaft rotates once every 4 seconds, so that changes in the temperature of the water, when necessary, are made that often, giving practically continuous regulation. With three different degrees of automatic adjustment in either direction, and with the possibility of shifting the slider by hand, the water flow may be quickly brought to any desired temperature and easily maintained. This device for causing the movement of the contact on the rheostat is shown in Plate XXXV, figure 2, which shows also the rheostat and the motor for adjusting the rheostat.

The indicator on which the desired temperature is set, which may be seen in Plate XXXVI, figure 2, at the right of the water heaters, is a special Wheatstone bridge, in one arm of which is the resistance thermometer in the water channel. The resistance of the slide wire of this bridge, which is nearly 45 cm. long, is sufficient to compensate for an upsetting of the balance of the bridge due to the change in resistance of the thermometer that would result from a change of 10 degrees in the temperature of the water, and by means of a coil in series with the wire the amount of balance resistance may be doubled. When the coil and slide wire are in series, the range of the dial is from 12 to 24 degrees;

when the coil is not in series, the reading is from 2 to 12 degrees. In each degree there are 10 subdivisions which are about 4 mm. apart, so that it is possible to change the setting of the indicator by as little as 0.05 degree, and the galvanometer connected with the indicator is sensitive to a change of this magnitude in the balance of the bridge. The resistance thermometer which is in one arm of the bridge is a coil of platinum wire, of approximately 25.5 ohms' resistance at 20° C., the resistance of which changes 0.1 ohm with a change of 1 degree in its temperature. The wire is wound in a flat, narrow coil and inclosed in a very thin silver case, resembling that of the heating coil, and similarly mounted in the upper end of the water channel. The resistance wire very rapidly acquires the temperature of the water flowing in the millimeter space surrounding the case, and changes in resistance instantly follow very small changes in the temperature. The thermometer is a short distance from the bridge, as shown in Plate XXXVI, figure 2, and connected with it by leads that are compensated so that the effect of the resistance of the leads and of change in their resistance due to change in temperature is eliminated.

From the final heater the water flows into a bottle of about 1-liter capacity, nearly full of broken pumice, and then into the heat absorber.

MEASURING THE TEMPERATURE INCREASE IN HEAT ABSORBER

The water that has passed through the heat absorber will have increased in temperature according to its rate of flow and the rate of production of heat in the chamber. The accuracy with which the increase in temperature is determined is of fundamental importance in the measurement of heat generated.

MEASUREMENT BY MERCURY THERMOMETERS

The difference between the temperature of the ingoing and that of the outgoing water was formerly determined by reading two mercury thermometers installed in the water circuit, with the bulb of one in the water just entering the chamber and the bulb of the other in the water just leaving it. The thermometers were as sensitive as it was practicable to employ and were very accurately calibrated. Each had a range of about 12 degrees, with graduations of 0.02 degree, the one in the ingoing water reading from 0° to 12° and the one in the outgoing from 8° to 20°, and by judging the position of the mercury between the graduations the temperature was estimated to 0.01 degree. The observer read the thermometers and recorded the temperatures every two or four minutes, which, in addition to the other duties at the observer's table, was rather tedious and trying. Both thermometers were supposed to be read simultaneously, but as this was impracticable for one observer the two thermometers were read as quickly as possible, and then the

observations were recorded. This method afforded opportunity for errors in fundamental data, some of which might be obvious, but most of which would not be detected.

MEASUREMENT BY ELECTRIC-RESISTANCE THERMOMETERS

To relieve the observer of the tedium of these observations, and especially to eliminate as much as possible of the personal element from the measurement, the mercury thermometers were replaced by a device for measuring the increase in the temperature of the water by the difference in electrical resistance of two coils of wire in the water circuit. Atwater and Rosa (4, p. 25; 5, p. 151) employed a device of this character in their original calorimeter, but did not develop it to measure temperature differences with the same degree of accuracy as the one here described. The latter device comprised two special resistance coils, a special Kohlrausch bridge, a sensitive galvanometer, and a lamp and scale for reading the deflections of the galvanometer. The specially mounted resistance coils, called the "bulbs," were inserted in the water line where the bulbs of the mercury thermometers had been and were connected with the special Kohlrausch bridge on the observer's table, the two coils being in opposite branches of the bridge circuit, with the slide wire between them. The reflecting D'Arsonval galvanometer by which the bridge was shown to be balanced was suspended in such a position that the scale on which the deflections of the galvanometer were read was on a level with and directly in front of the eyes of the observer sitting at the table. The movement of the galvanometer was indicated by the movement of a vertical line of light along the scale, the light from a straight-filament electric lamp being reflected by the mirror of the galvanometer. To determine the difference in the temperature of the two coils, it was merely necessary to move the battery circuit contact along the slide wire of the bridge until the line of light was at the center of the scale, showing that the bridge was balanced. The reading of the bridge scale was then recorded. To balance the bridge and read its scale was much more convenient than to read the mercury thermometers, and only one record was involved.

Several types of resistance-thermometer bulb were tried in connection with this device. In one, insulated resistance wire was incased in a coil of small-bore lead tube, which was immersed in an enlargement in the water channel. This proved unsatisfactory for several reasons. One was that it did not respond quickly enough to changes in the temperature of the water, owing probably to poor thermal contact between the wire and the tube; and the mass of metal in the tube also tended to increase the lag. Another was that the space in the lead tube was not deprived of water vapor, and this eventually moistened the insulation of the resistance wire, so that a short circuit was established between the wire and the

tube sufficient to ruin the bulb for accurate measurement of temperature. After the bulbs had been in use a short time they would produce an electromotive force as if they were primary or secondary cells.

In another type of thermometer bulb the resistance wire was inclosed in a thin-walled small-bore copper tube, which was filled with Wood's metal to exclude moisture from the tube and to render the thermometer more sensitive by increasing the conduction of heat to the wire. This bulb did not prove satisfactory because, though the thermal conductance from the water to the wire may have been improved, the sensitiveness of the thermometer was not, the mass of metal apparently causing a lag in response to temperature change. Furthermore, the Wood's metal apparently did not completely exclude moisture, for ultimately the wire in this thermometer also became short-circuited with the metal. Another serious objection was the possibility that the resistance wire might be stretched by the unequal expansion of the metal in which it was embedded.

The bulb which was finally used with utmost satisfaction was constructed in accordance with the specifications of the one developed by Dickinson and Mueller (11, 12, 13) in connection with investigations on calorimetry at the United States Bureau of Standards, which was designed especially for use in determining the temperature at a definite point of liquid flowing in a tube in a continuous-flow calorimeter. The bulbs were designed especially to combine constancy, freedom from lag, and intimate contact with the entire water flow. The platinum resistance wire was wound on a thin strip of mica, and this coil, laid between two similar mica strips, was inclosed in a flat sheath of thin silver which pressed the mica insulating strips firmly against the resistance wire, thus affording opportunity for rapid conduction of heat between the case and the wire. The silver case terminated at the top in a tube which was sealed to a glass tube, on the end of which was a bulb containing phosphorus pentoxid, the purpose of which was to exclude moisture from the space in which the resistance wire was inclosed. The flat part of this bulb, which was about 10 cm. long, 10 mm. wide, and 1 mm. thick, and contained the sensitive part of the thermometer, was inserted in a brass tube with a constricted channel, like that for the final heater described on page 319, so that the sensitive portion of the thermometer was surrounded by a space about 1 mm. across; and water flowing through this space was thus brought into intimate contact with the thermometer, which very rapidly acquired the temperature of the water and responded instantly to changes in temperature and integrated stream lines of temperature, if any existed. The two thermometers, one in the ingoing and the other in the outgoing water, had exactly the same resistance, about 25.5 ohms at 20° C., and the same coefficient of change of resistance with change in temperature, about 0.0039 per degree for the range of temperature in which they would be used, the resistance change of each thermometer being 0.1 ohm per

degree. With the regular leads to each thermometer from one branch of the bridge circuit was a compensating loop from the opposite branch of the bridge, to balance the resistance of the leads in both branches of the circuit, and to eliminate the effects of changes in the resistance of the leads due to changes in their temperature and of thermal electromotive forces. All connections in the bridge circuit were soldered—that is, there were no contact connections; hence, no possibility of error due to contact resistance in any part of the circuit.

The special Kohlrausch bridge was designed to measure any difference as large as 10 degrees or as small as 0.01 degree in the temperature of the water as it entered and as it left the heat absorber. The slide wire of the bridge, which was about 4.5 meters long, consisted of 10 turns of manganin wire wound spirally on a cylinder of marble about 15 cm. in diameter. The battery-circuit contact, which balanced the bridge by the adjustment of its position on the slide wire, was mounted on the inside of a hood surrounding the cylinder, which, when rotated, moved up or down on a threaded center post. Since the contact was in the battery circuit, whatever contact resistance there might be had no effect on the balancing point of the bridge. The resistance of the total calibrated portion of the slide wire was sufficient to balance the bridge when the resistance of the two thermometer coils differed by as much as 1 ohm, which would occur with a difference of 10 degrees between the temperature of the ingoing and that of the outgoing water. With one rotation of the hood the contact was moved over sufficient of the slide wire to balance a difference of 0.1 ohm or 1 degree in the thermometers. On the edge of the hood was a scale with 200 divisions, each corresponding to a little over 2 mm. of the slide wire. A movement of the contact on the wire the space of two divisions would be sufficient to balance a difference of 0.001 ohm or 0.01 degree in the thermometers.

The sensitivity of the galvanometer was sufficient to indicate a change of even one division in the bridge setting, equivalent to 0.005 degree in the temperature of the thermometer. With the usual current of 0.03 ampere in each half of the bridge, a change of 0.001 ohm would be indicated by a deflection of several millimeters on the galvanometer scale. A current of 0.03 ampere flowing in each resistance thermometer would not cause an increase of 0.005 degree in the temperature of either, when immersed in water flowing at the rate of 200 c. c. per minute, which would be not over half the common rate in the experiments.

Provision was made for checking the results obtained with the electric-resistance thermometers. The second type of resistance bulb mentioned above was constructed so that the bulb of the mercury thermometer formerly used could be inserted into the bulb of the resistance thermometer, and the temperature differences determined by both sorts of thermometers at the same time. The results obtained by the two

methods before the resistance coils became short-circuited were always in very satisfactory agreement, but this was hardly a sufficient test of the accuracy of the resistance method, because the measurement of temperature difference by the electric-resistance thermometers is much superior to that by the mercury thermometers in sensitivity and precision. With the third type of resistance bulb a more satisfactory method of checking was provided. A differential thermoelement, with several junctions of copper and constantan wire in each end inclosed in thin glass tubing, was mounted with one end in the water just leaving the ingoing thermometer and the other end in the water just entering the outgoing thermometer. The terminals of the thermoelement were connected with binding posts on the observer's table, from which connection could be made with a potentiometer, by means of which temperature differences could easily be measured to an accuracy of 0.01 degree. Measurement of the increase in temperature of the water flowing in the heat absorber by means of this apparatus afforded a real check on the measurement with the resistance thermometer.

MEASUREMENT BY TEMPERATURE DIFFERENCE RECORDER

As a matter of fact, this method of measurement could be employed instead of the resistance-thermometer method when the readings are to be made and recorded by the observer. Either method was more convenient and decidedly more sensitive than the mercury thermometers, and by use of it the temperature difference was actually measured to 0.01 degree, whereas in reading the mercury thermometers the temperature was only estimated to 0.01 degree. The particular advantage in the resistance thermometers was in the opportunity to use with them a device which gives automatically a practically continuous record of the difference between the temperature of the water entering and that of the water leaving the heat absorber. A device of this character which has been employed for five years in the investigations with the present respiration calorimeter has proved very satisfactory indeed and relieves the observer of a considerable amount of drudgery, while it entirely eliminates the possibility of error due to personal inaccuracy in recording data regarding the temperature differences.

Like the resistance thermometers described above, the two coils used in this device have the same resistance, approximately 25.5 ohms, at the same temperature, and the same change in resistance with the same change of temperature, but the bulbs differ somewhat in mechanical construction from the earlier type. The platinum resistance wire is not in a thin, flat coil in a flat sheath, but is in a helical coil in a narrow annular space between two metal tubes with thin walls. The wire is wound upon the inner tube, and the outer tube fits close against it, an electrical insulation of thin sheet mica separating the wire from each tube. The space between

the tubes is tightly closed at each end, the leads from the resistance wire being carried out through a small tube attached to the tube surrounding the wire. As in the flat-type thermometer, this small tube terminates in a bulb containing phosphorus pentoxid, to keep the annular space free from moisture. The cylindrical shell inclosing the resistance wire is mounted in a brass tube which provides a water channel so designed that the water flowing in it passes inside the inner and outside the outer of the tubes incasing the wire, which is thus brought into intimate contact with all the water flowing through the thermometer, and responds instantly and accurately to changes in its temperature. Because of the design of the thermometer and the manner in which it is mounted in the walls of the chamber, the usual fluctuations of the temperature of the air adjacent to the case of the thermometer introduce no error in the measurement of the temperature of the water flowing in the bulb. One of these thermometers is placed in the incoming water pipe so that it will be at the temperature of the water just as it passes through the copper wall, and the other is similarly placed in the outgoing water pipe.

The two thermometer coils are in the corresponding arms in opposite branches of the circuit of a special Wheatstone bridge (Pl. XXXV, fig. 2), which may be accurately balanced for inequalities in resistance of the coils as small as 0.001 ohm and as large as 1 ohm, resulting from a difference of 0.01 degree and of 10 degrees, respectively, between the temperature of the water entering and that of the water leaving the heat absorber. The total resistance of the slide wire of the bridge will compensate for an inequality of 0.2 ohm in the resistance of the coils which results from a difference of 2 degrees in their temperature. If there is no difference in the temperature of the water in the two thermometers, the bridge is balanced with the battery circuit contact at the low end of the wire, while if the temperature of the water leaving the heat absorber is 2 degrees higher than that of the water entering it, the bridge is balanced when the contact is at the upper end of the wire. To compensate for inequalities due to temperature differences greater than 2 degrees, eight coils of manganin wire in series are arranged so that any number of them may be connected in series with the slide wire, thus shifting the position of the contact on the wire at which the bridge may be balanced and altering the significance of the balance point in temperature difference. The lower end of the wire may thus be made to correspond to any whole number of degrees of temperature difference between 0 and 8, with the upper end 2 degrees higher in each case. The coil and slide wire are joined by means of a heavy copper link, with one end in the mercury cup in which one end of the slide wire terminates and the other end in a similar cup in which an end of the extension coil terminates.

The slide wire of the bridge is incorporated in a mechanism (Pl. XXXV, fig. 2) which automatically balances the bridge for inequalities of resist-

ance in the thermometer coils and records the balancing operations in terms of temperature difference and time. The wire is mounted in a bar which supports and guides a slider carrying the battery circuit contact point along the slide wire. The slider is actuated by a small electric motor, the direction and extent of motion of the slider being governed by the direction and the amplitude of deflection of the pointer of a galvanometer which is connected between the two branches of the bridge circuit, and is incorporated with the slide wire in the mechanism which balances the bridge. The direction in which the pointer will swing depends upon whether the inequality of resistance of the thermometer coils increases or decreases—that is, whether the difference between the temperatures of the water in the thermometers grows larger or smaller. For example, if the temperature of the outgoing water rises or that of the ingoing water falls, the pointer will swing so as to cause the slider to move toward the high end of the wire. The amplitude of deflection of the pointer depends upon the magnitude of the inequality of resistance of the thermometer coils. The bridge and galvanometer are sensitive to very small temperature changes in the thermometer. With the measuring current of 0.05 ampere in each thermometer coil a difference of 0.0005 ohm in the resistance of the two coils, which results from a difference of 0.005 degree in the temperature of the water in the thermometer, causes a deflection of the pointer sufficient to influence the position of the contact on the slide wire. With a measuring current of 0.05 ampere each coil would dissipate about 0.06 watt, which would be sufficient to raise the temperature of the thermometer 0.005 degree if the water were flowing through it at a rate of only 200 c. c. per minute; but since the rate of flow is generally twice as great, the effect of the measuring current on the temperature of the bulb is negligible.

Each time it changes the position of the battery circuit contact point on the slide wire the automatic shifting mechanism moves the slider one of three different distances in either direction, according to the amplitude of deflection of the galvanometer pointer. With the smallest change of position the contact is moved along the wire sufficiently to balance the bridge for inequality of resistance in the thermometers due to differences of less than 0.01 degree in the temperature of the water. The medium change balances differences of resistance equivalent to differences of nearly 0.03 degree in temperature, and the large change corresponds to temperature differences of 0.05 degree. The shifting mechanism functions every 7 seconds; hence, it will keep the bridge in balance for any change in temperature difference not exceeding 0.4 degree per minute; but inasmuch as the position of the contact point on the slide wire may be easily adjusted by hand for any inequality of resistance within the range of the instrument, any alteration in temperature difference may be followed.

As the slider moves back and forth on the bar which supports the slide wire, it carries a pen which draws a curve on ruled paper by which the movement of the contact point on the slide wire is expressed in temperature. The total width of the paper scale, 25 cm., represents a difference of 2 degrees between the temperature of the water entering and that of the water leaving the heat absorbers, and corresponds exactly to the length of the slide wire by which the bridge is balanced for the inequality of resistance in the thermometer coils resulting from such a temperature difference. The temperature difference indicated by the position of the pen on the paper scale coincides with that to which the position of the contact point on the slide wire is equivalent. The paper scale is ruled with 100 lines, each representing 0.02 degree, and as the distance between the lines is 2.5 mm., the curve may easily be interpreted to 0.01 degree. The paper is moved forward at a very regular rate, approximately 7.5 cm. per hour, by the motor which moves the slider, the speed of the motor being regulated by a governor so that it is uniform, even with wide fluctuations in voltage of the current by which the motor is driven. Since the necessary changes in the position of the slider are made every 7 seconds, the curve gives a practically continuous record of the temperature difference.

The difference between the temperature of the water as it enters and that as it leaves the heat absorber may thus be easily read at any instant to 0.01 degree. The accuracy of the measurement of temperature difference by the apparatus may be tested at any time, even during the course of an experiment, without interfering with the record, and such tests are made at frequent intervals. In the water channel in the center of each resistance-thermometer bulb is the end of a differential thermoelement of 0.125 mm. copper and constantan wires, having 11 junctions in each end, inclosed in 4-mm. glass tubing, with thin wall. The element remains permanently in position, though it may be easily removed if necessary. The terminals of the element are joined by insulated 1-mm. copper wire to binding posts on the observer's table, from which connection can be made with a potentiometer whenever a test is to be made. With this differential thermoelement, which has been calibrated over a wide range of temperature at the United States Bureau of Standards, an electromotive force of over 4.5 microvolts results from a difference of 0.01 degree in the temperatures of the two ends. By means of the potentiometer and galvanometer with which it is employed, an electromotive force of half that magnitude is easily measured; consequently temperature differences may be measured by it to an accuracy at least as good as 0.01 degree. Measurements made with this apparatus therefore serve to indicate the accuracy of those with the recorder. The agreement of results obtained by the two methods of measuring the increase in the temperature of the water flowing through the heat ab-

sorber is shown in Table I, which summarizes data obtained in an alcohol check test (see p. 342) of the calorimeter made in January, 1915, which continued for two consecutive periods of three hours each.

TABLE I.—Comparison of data for heat measurement obtained by use of temperature difference recorder and of thermoelement with potentiometer

Time.	Water flow.	Temperature difference.		Heat computed from measurement.	
		By recorder.	By potentiometer.	By recorder.	By potentiometer.
	<i>Kgm.</i>	<i>Degrees.</i>	<i>Degrees.</i>	<i>Calories.</i>	<i>Calories.</i>
1 hour.....	20.53	3.99	4.01	81.9	82.3
Do.....	21.20	3.97	3.96	84.2	84.0
Do.....	23.00	3.73	3.73	86.0	86.0
Total.....				252.1	252.3
1 hour.....	23.00	3.68	3.68	84.6	84.6
Do.....	23.21	3.57	3.54	82.9	82.2
Do.....	22.72	3.67	3.67	83.4	83.4
Total.....				250.9	250.2

In order that the recording device may continue to measure temperature differences with the accuracy required, not only must the bridge be sensitive to a change as small as 0.002 per cent in the resistance of the thermometer coils, but also the resistances of the various parts of the bridge circuit other than the thermometers must not change as much as 0.003 per cent. Provision is made for testing the component parts of the bridge by the substitution of duplicate parts, which are mounted with the ratio coils of the bridge in a check box, and tests of this character are made at frequent intervals. After the apparatus had been in use for a short time a very slight change in one of the ratio coils was detected and corrected. Since that time the bridge has remained remarkably constant. It is possible also to test with the check box and recorder whether the thermometer coils remain alike in resistance at the same temperature. Provision is made in the check box for correcting slight inequalities in them by a variable shunt across a coil of small resistance in series with one of the thermometers.

PREVENTING TRANSFERENCE OF HEAT THROUGH THE WALLS OF THE CHAMBER

In order that the quantity of heat produced in the chamber may be accurately measured, either there must be no increase or decrease in it due to the passage of heat through the walls of the chamber, or if heat is thus added or subtracted, the quantity must be determined and allowance made for it. This calorimeter is constructed and operated in accordance with the method employed in the original calorimeter of

Atwater and Rosa, to prevent gain or loss of heat through the walls, though with modifications in details which make the present apparatus exceedingly sensitive, while easy to operate. The copper wall¹ of the chamber is duplicated by a wall of zinc attached to the outside of the iron framework which supports the copper wall, as explained on page 303, and the temperature of the zinc wall is regulated to accord with that of the copper wall in such manner that the thermal conditions of the two walls will be in equilibrium with each other. When the temperature of the zinc wall is the same as that of the copper wall, the quantity of heat transmitted from each wall to the other is the same, so that neither wall actually gains heat from the other. The effect of such a condition on the quantity of heat in the chamber would be the same as if no heat were to pass from either wall to the other in either direction. If the temperature of the zinc wall is above that of the copper wall, the quantity of heat passing from the zinc to the copper is greater than that in the reverse direction—i. e., the copper wall will gain heat from the zinc wall, some of which, at least, it will transmit to the air of the chamber. Conversely, if the temperature of the zinc wall is below that of the copper wall, the former will gain heat from the latter, some or all of which the copper wall has derived from the air of the chamber. If the quantity of heat which the copper wall has gained from the zinc wall is counterbalanced by an equal quantity gained by the zinc wall from the copper wall, the effect on the measurement of the quantity of heat produced in the chamber is the same as if no heat had been transferred from either wall to the other. This counterbalancing may be accomplished by keeping the temperature of the zinc wall above or below that of the copper wall, as need be, to the same degree and for the same length of time that the conditions were reversed. For this purpose means are provided for determining when the zinc wall is warmer or colder than the copper wall, and for heating and cooling the zinc wall as is found necessary.

DETECTING DIFFERENCES IN TEMPERATURE OF THE DOUBLE METAL WALLS

Thermoelectric thermometers are used to detect any difference between the temperature of the zinc wall and that of the copper wall. Differential thermoelements are installed between the two walls, with the junctions at one end of each element close to the outer surface of the copper wall, while those of the other end are in the plane of the zinc wall, and the terminals of the elements are connected with a sensitive galvanometer. The direction of the deflection of the galvanometer indicates whether the zinc wall is warmer or cooler than the copper wall—i. e., whether to warm or to cool the zinc wall.

Each thermoelement consists of four copper-constantan couples made of bare hard-drawn wire about 1 mm. in diameter (No. 18, American gauge). In making the junctions, the copper and constantan wires were put end

¹ As used in this section, the term "wall" may include the ceiling and the floor as well as the side walls.

to end and joined with silver solder. The wires were then bent at the junctions into a grid, with the parallel lines about 5 mm. apart and with copper and constantan alternating. Each constantan wire and three of the five copper wires are about 7 cm. long, so that the distance between the two opposing sets of junctions is the same as that between the copper wall and the zinc wall. The two other copper wires, which are at opposite ends of the series, are longer, to form leads for the element, as explained below.

Wire of the size stated was used chiefly because it was most readily available and seemed quite well adapted to the type of element constructed. Theoretically, a small wire would be preferable, because of smaller thermal conductance, but the support in which each element is mounted probably greatly delays change in temperature of the wires between the junctions, while affording opportunity for rapid change at the junctions. This support consists of a hard maple rod or spindle about 10 cm. in length and 15 mm. in diameter. A recess 8 mm. wide and 2 mm. deep is cut around the spindle 3 cm. from one end, and in the surface are 10 equally spaced longitudinal slots, each nearly 1 mm. wide and 2 mm. deep. The five copper and four constantan wires which, joined alternately in series in a grid, as described above, comprise the four differential thermocouples of an element, were forced into these slots until they were about a millimeter below the surface of the wood and to that extent were protected against contact with the metal sleeve and thimble by which the thermoelement is supported in the walls, as explained below. By means of a cut between two adjoining slots near the center of the spindle the copper wire at one end of the series is doubled back and extends parallel with the copper wire at the other end of the series, the two projecting from one end of the spindle and providing terminals for the element. The spindles with the wires thus embedded were boiled in paraffin for two or three hours, so that they would not swell or shrink with changes in the humidity of the air.

The temperature of the wires thus embedded in the spindle is probably that of the spindle and therefore changes slowly—i. e., the temperature gradient in each wire is quite like that of the others in the element and is relatively constant for considerable periods. On the other hand, the junctions between the copper and the constantan wires are not embedded, one series of four alternate junctions projecting into the air at one end of the spindle, while the series of opposing junctions projects into the air in the recess near the other end of the spindle, so that changes in the temperature of the air surrounding them affect the junctions quickly.

To keep each element in place between the two metal walls a short copper sleeve is passed through a hole in the zinc wall, the sleeve being soldered to the zinc at the edge of the hole to insure good thermal conductance; and directly opposite, with its open end facing that of the sleeve, a short copper thimble is firmly soldered to the outer surface of

the copper wall. A spindle is pushed through the sleeve and into the thimble until the junctions projecting from its inner end are very close to the bottom of the thimble, actual contact being prevented by the adjustment of a small screw in the end of the spindle. A change in the temperature of the copper wall immediately affects the temperature of the thimble attached to it, and consequently that of the junctions within the thimble. The junctions in the recess at the other end of the spindle are within the sleeve attached to the zinc wall, and any change in the temperature of the zinc wall affects the sleeve and, hence, the temperature of the junctions within it. Since both the sleeve and the thimble are short, neither affects the temperature of the wire in the elements any considerable distance from the junctions. The sleeve, however, projects slightly either side of the zinc wall, so that it will surround the junctions, even when they might come inside or outside the plane of the zinc, because of inequalities of distance between the two metal walls.

A short section of the spindle, between the recess and one end, projects from the outer end of the sleeve in the zinc wall and provides a firm stay for the terminals of the elements.

There are 95 such thermoelements distributed in the walls of the chamber. If they were equally spaced there would be one for each 4.5 dm. square of surface; but since the temperature of the chamber would tend to vary more at the top than at the bottom, more elements were installed in the upper than in the lower parts of the chamber to increase the sensitivity and integrate a larger number of sections of the walls. There are accordingly 16 elements in the ceiling and 10 in the floor. In the sides are five rows, with 14 elements in each row except the first one from the top, from which one is missing because the space in which it would be located is occupied by the window. The five rows are not quite equally separated, the two upper rows being slightly nearer together than the three lower ones, in accordance with the idea that the temperature of the upper section would tend to vary more than that of the lower one. These thermoelements are joined in groups in such manner that a difference between the temperature of the copper wall and that of the zinc wall may be detected in certain portions of the walls without regard to conditions in other parts. One group includes the 16 elements in the top; another the 28 elements in the two upper rows of the sides, called the upper zone; a third, the 42 elements in the three lower rows of the sides, called the lower zone; and a fourth group, the 10 elements in the bottom. The thermoelements in each group are connected in series by heavily insulated No. 18 copper wire, and the same sort of wire leads from the terminals of each group to a multiple point switch on the observer's table by which the groups may be connected successively with the galvanometer. It is also possible to connect all 95 thermoelements in series as a whole with the galvanometer and thus observe the average difference between the temperature of the copper wall as a whole and that of the zinc wall as a whole.

In the multiple-point switch the leads from the different groups of thermoelements terminate in a double row of studs arranged in segments of concentric circles, and the galvanometer leads terminate in two metal rings concentric with the studs (Pl. XXXIII, fig. 1). Metal strips, passing through a vertical shaft at the center of the circles, complete the circuit from studs to rings, the ends of the strips being bent to touch edgewise. On turning the shaft by means of the handle at the top, the strips are shifted from one pair of studs to another, thus connecting the different systems with the galvanometer. The switch includes studs not only for the thermoelement groups described above, but also for resistance thermometers described beyond, so that the same galvanometer will serve for several systems.

The galvanometer with which the electromotive forces in the thermoelement circuits are detected is a reflecting instrument of the D'Arsonval type, with a coil resistance of 39 ohms. When critically damped, it has a period of 7 seconds, and a sensitivity such that an electromotive force of approximately 2 microvolts in either circuit will cause a deflection of 1 mm. on a scale 1 meter from the mirror of the galvanometer.

With this galvanometer the number of thermoelements in each circuit is sufficient to cause a fairly large deflection when the temperature of the zinc wall is only slightly different from that of the copper wall. In the bottom section, for example, there are 10 thermoelements, the smallest number in any section, each with four differential couples, and each couple having a thermal electromotive force of close to 40 microvolts per degree of temperature difference between the junction at one end and that at the other. All 40 couples being in series, there would be a total electromotive force of 1,600 microvolts for an average difference of 1 degree between the temperature of the copper wall and that of the zinc wall in this section, or 16 microvolts for an average difference of 0.01 degree. Since an electromotive force of about 2 microvolts will cause a deflection of 1 mm., a difference of 0.01 degree would cause a deflection of at least 7 mm. It is easy to read a deflection of less than 1 mm.; consequently the effect of a temperature difference as small as 0.001 degree between the junctions at opposite ends of the thermoelements in this may be observed. The effect of such a difference in the other sections would be greater, because of the larger number of elements; the 16 in the top would cause a deflection of more than a millimeter; the 27 in the upper zone of the sides about 2 mm.; and the 42 in the lower zone more than 3 mm.

CONTROLLING THE TEMPERATURE OF THE ZINC WALL OF THE CHAMBER

The temperature of the zinc wall is raised or lowered by heating or cooling the air confined in the narrow space adjacent to the outer surface of the zinc, which has a corresponding effect on the wall. Parallel with the wall, and about 4 cm. outside of it, is a wall of cork board 38 mm. thick,

which is such a good heat insulator that appreciable changes in the temperature of the air in the laboratory affect the temperature of the air confined in the spaces between the cork board and the zinc wall very slowly. The temperature of the air in this space adjacent to the zinc wall is raised by converting electrical energy into heat in a resistance wire that is strung on porcelain insulators attached to the wall; and it is lowered by passing cold water through small-bore brass pipes supported by small iron hooks screwed to the framework to which the wall is attached. Short sections of both pipes and wires and the method of attaching them to the wall are shown in Plate XXXIV, figure 2.

By wooden strips extending from the metal wall to the cork board, the air space surrounding the zinc wall is divided into sections corresponding with the top, the upper and lower zones of the sides, and the bottom of the chamber, as already described in the case of the thermoelements in the walls. A portion of one strip is shown in Plate XXXIV, figure 2. Each section has its own heating device and cooling device, so that the temperature of the corresponding portion of the zinc wall may be controlled independently of the conditions in any other space, and the possibility of heat entering the chamber in one part of the wall and leaving it in another is prevented.

The current of water for cooling the zinc wall flows through brass pipe of about 7 mm. bore (so-called $\frac{1}{8}$ -inch pipe). In the top and bottom sections the pipe extends forward and back from end to end for the whole width of the space, the successive lengths of pipe being about 15 to 20 cm. apart. In the upper and lower zones the pipe extends continuously around the four sides of the walls, the succeeding turns of the coil being about as far apart as those in the other sections. This furnishes ample cooling effect, which can be regulated by varying the temperature of the water flowing in the pipe, or the rate of flow, or both. The inlet ends of the four pipes are connected with the feed-water pipe, with the small brass needle valves used for regulating the flow in the cooling coils close together and convenient to the operator at the observer's table (Pl. XXXVI, fig. 2). The outlet ends of the coils are also brought together in a funnel below the regulating valves, so that the effect of the valves on the rate of flow may easily be seen.

The electric current for heating the zinc wall is conducted by a non-corrosive wire of a high carrying capacity, the resistance of which is about 3.5 ohms per meter. In each space the wire is distributed, as the cooling coils are, over practically the whole surface of the zinc, the successive lengths of wire extending from one end of the space to the other, about 15 cm. apart. The amount of wire in each space is such that without regulation of the current in it the heating effect would be greater than necessary. With the proper ballast resistance in series with each heater the heating effect in each section may, if desired, be made proportional to the area of zinc to be heated. In the upper zone

of the sides, for example, there is an area of about 5.8 square meters. The total resistance of the wire in the space is 143 ohms. In series with this wire but exterior to the space is a resistance unit that may be varied according to the need for current. If a unit of 200 ohms' resistance were used, there would be a little over 0.64 ampere of current flowing in the heating wire, the pressure of the current being 220 volts; and the total amount of electrical energy (I^2R) dissipated in the 143 ohms of wire would be nearly 59 watts, or roughly 10 watts per square meter of surface of zinc. Similarly, the area of the lower zone is about 8.9 square meters, and the resistance of the wire in it is 195 ohms; with an exterior unit of about 125 ohms in series with the heating wire, the amount of energy dissipated in the latter would be about 92 watts, or slightly over 10 watts per square meter. There are close to 2.9 square meters in the top section and the same area in the bottom, and in each of these sections is a heating coil of 117 ohms; with an exterior unit of 325 ohms in series with it, the current in each heater would approximate 0.5 ampere, and about 29 watts would be dissipated in the 117 ohms of resistance wire, or 10 watts per square meter.

In controlling the temperature of the zinc wall cold water is kept flowing continuously through the brass pipe in the air space outside of it at such a rate of flow, depending upon the temperature of the water, that the temperature of the unheated air would be lower than that at which the wall is to be kept. With a constant flow of water the temperature gradient along the pipe is quite flat in comparison with what it would be if the rate of flow were increased or decreased as the air would need to be cooled or heated; in other words, the cooling effect is fairly uniform throughout the length of the pipe. At the same time electric energy is converted into heat in the resistance wire until the air is warmed enough to bring the wall to the desired temperature. Since this dissipation of heat is equal in all parts of the wire, the total mass of air in the space is quite uniformly heated. Under these conditions to change the temperature of the wall requires only an increase or decrease of the current in the resistance wire, according to whether the wall is to be heated or cooled, which involves merely the adjustment of a rheostat in series with the wire, so that regulation is easily and quickly effected. A rheostat of oxidized constantan wire wound on an enameled metal tube and having a sliding contact passing over successive turns of the wire, with a resistance of about 980 ohms and a current-carrying capacity of 1 ampere, is in series with the resistance wire comprising the heating coil in each section. The four rheostats for the different air sections to be controlled are attached vertically to an asbestos slab at one end of the observer's table, as seen in Plate XXXVI, figure 1, with the sliding contacts in easy reach of the operator reading the galvanometer deflections.

The temperature of the zinc wall is kept as nearly as possible like that of the copper wall, so that the deflections of the galvanometer connected with the differential thermoelements in the walls are as close as possible to 0. Even under the most favorable conditions it is hardly practicable to keep the two walls so uniformly alike that there will be no deflection at any time, because the temperature of the copper wall, however well regulated, does vary to some extent, and it is not possible to anticipate the change. It is possible, however, to keep the deflections most of the time so small that any error introduced by the temperature differences which they indicate would be insignificant. As explained on page 334, the number of thermoelements in each section of the walls and the sensitivity of the galvanometer are such that a very small difference between the temperature of the copper wall and that of the zinc wall would cause a fairly large deflection; hence, a very small deflection really means a practical identity of temperature of the two walls. When the rate of production of heat within the chamber is quite uniform and the rate of abstraction of heat is so nearly like it that the temperature conditions within the chamber are quite constant, the temperature of the zinc wall may be kept so nearly like that of the copper wall that the deflection will not exceed 5 mm. and will generally be less. A deflection of that magnitude would indicate for the bottom section a difference not greater than 0.005 degree between the average temperature of the copper floor of the chamber and that of the zinc wall outside of it; for the other sections it would indicate still smaller differences. The amount of heat gained by either wall from the other with such small differences is of little importance in comparison with the total amounts usually measured in the chamber. In an experiment with a variable heat production, as would be the case with a man moving and quiet by turns, such a close balance could hardly be maintained at all times, though the deviation need not greatly exceed 5 mm. for any considerable periods. Furthermore, it is possible to make the deflections in one direction equal to those in the other direction for equal short periods, so that whatever heat may be gained by the copper wall from the zinc wall during one period is counterbalanced by that gained by the zinc wall from the copper wall during the succeeding period, in which case there is no actual increase or decrease of the quantity of heat in the chamber for the total time of the two periods due to an exchange of heat between the walls.

In order that the walls controlled in the manner described shall be heatproof, their temperature and that of the iron structure between them must be the same. The temperature of the copper wall, and consequently that of the zinc wall, is governed by that of the air in the chamber; but the two walls may be brought into thermal equilibrium at a temperature above or below that of the framework, in which case the quantity of heat in the chamber would probably be affected by the

mass of iron with its large thermal capacity and high conductivity, the magnitude of the effect depending upon the difference between the temperature of the iron and that of the air in the chamber. To avoid error from this source in the measurement of the heat generated in the chamber it is very essential not only to keep the temperature of the walls of the chamber and that of the air of the chamber as nearly alike and as constant as possible during the period in which the measurements are made, but also to be certain that at the beginning of the period the temperature of the iron structure is identical with that at which the walls and air are to be kept. To this end the regular experimental period must be preceded by a period in which the walls and their supporting structure are brought to the desired temperature. The length of this period depends upon the temperature conditions of the walls when it begins, but it is shortest when the temperature of the walls and framework is kept under control by means of a thermoregulator in the chamber during the periods in which experiments are not in progress. With care and attention to the details outlined it is possible to prevent gain or loss of heat through the walls of the chamber, but the amount of attention and manipulation necessary to avoid error because of the metal would be avoided if the framework were constructed of material having small thermal capacity and poor conductivity. Such a change would be made in reconstructing the calorimeter.

PREVENTING GAIN OR LOSS OF HEAT IN THE AIR ENTERING AND LEAVING THE CHAMBER

Provision is also made against loss or gain of heat in the circulating air. A thermoelement of 40 couples is installed with one end of each couple in the incoming air just as it enters the chamber and the other end in the outgoing air just as it leaves the terminals of the element leading to the multiple point switch on the observer's table, by which it may be connected with the galvanometer. Any difference between the temperature of the ingoing air and that of the outgoing air indicated by the galvanometer is corrected by heating or cooling the ingoing air as needed. A copper tube of small bore is coiled tightly on the brass pipe that conducts the air into the chamber for a distance of about 30 cm. just before the pipe enters the wall, and through this coil water runs continuously, tending to keep the air too cool. Adjacent to this, also on the brass pipe, is an electric heating coil of about 800 ohms' resistance, which warms the air to the desired temperature. To change the temperature of the air, only the current in the heating coil is varied. In series with this coil is a tube rheostat of about 2,500 ohms' resistance by which the current in the resistance coil and, hence, its heating effect are regulated, the position of the sliding contact being adjusted until the galvanometer indicates that the temperature of the ingoing air is the

same as that of the air leaving the chamber. This rheostat is mounted on the end of the observer's table beside those for controlling the temperature of the zinc wall.

ALLOWANCE FOR CONDITIONS AFFECTING THE HEAT OF THE CHAMBER

Any passage of heat into or out of the chamber through the walls or in the ventilating air current being prevented, the sum of the quantity of latent heat in the water vapor of the outgoing air and that of sensible heat removed by the water circulating in the heat absorber would equal that actually produced in the chamber if there were no change in the temperature of the walls or in that of any objects confined within them. Under ordinary conditions, however carefully the rate of abstraction of heat from the chamber has been regulated to accord with that of production, temperature changes can not be absolutely avoided, so they must be measured and allowance made for them.

CHANGE IN TEMPERATURE OF THE METAL WALLS

If the temperature of the copper wall is lower at the end of a given period than it was at the beginning, and the temperature of the zinc wall has been kept identical with that of the copper wall throughout the period, a certain amount of heat has been imparted to the air of the chamber by the copper wall during the period; or, conversely, if the copper wall is warmer at the end of the period, some heat has been absorbed from the air by the wall. To ascertain how much allowance must be made for the heat involved in such changes, it is necessary to determine the temperature of the copper wall at the beginning and the end of the period and to know how much heat is necessary to raise the temperature of the calorimeter a given amount—i. e., its hydrothermal equivalent.

The temperature of the copper wall is determined by means of an electric-resistance thermometer somewhat like that described on page 313 for determining the temperature of the air. In this thermometer, however, each of the six coils of nickel resistance wire is wound on a thin fiber strip about 12 cm. long and 1 cm. wide, and is covered with a thin layer of silk and lacquered, the completed bulb being about 1.5 mm. thick. A strip of brass, slid into guides soldered to the copper wall, presses each coil firmly against the wall so that there is close thermal contact with metal on each side of the coil; hence, changes in the temperature of the wall affect the resistance wire very quickly. These six coils, joined in series by well-insulated No. 16 copper wire, are distributed on the side walls and ceiling in such manner as to show the average temperature of the total mass of copper. The terminals of the series of coils are connected with the special switch, mentioned on page 334, and through that with the temperature indicator (Wheatstone bridge) on the observer's table. The bridge and galvanometer are sensitive to resistance changes

in the thermometer coils that would result from a change of 0.01 degree in the temperature of the copper wall.

The hydrothermal equivalent of the calorimeter has been estimated from determinations of the quantity of heat that had to be dissipated in the chamber to raise the temperature of the copper wall 1 degree, and the amount of heat that was imparted to the air of the chamber when the temperature of the copper wall fell 1 degree, while the thermal conditions of the zinc walls were kept in equilibrium with those of the copper wall during the change. The capacity for heat as determined in both ways was not far from 40 Calories. From the weights and specific heats of the materials entering into the construction of the chamber the hydrothermal equivalent was calculated to be between 35 and 40 Calories. According to these figures, the quantity of heat in the chamber should be increased by 40 Calories with a fall of 1 degree, or decreased by 40 Calories with a rise of 1 degree in the temperature of the copper wall, if the thermal conditions of the zinc wall were in equilibrium with those of the copper wall while the change occurred.

This will be the case, provided the change in thermal conditions has occurred in such manner as to affect the iron supporting structure the same as the copper wall. In constructing the calorimeter no provision was made for determining the actual temperature of the structure, the assumption being that the thermal conditions of the iron framework would also be controlled by the regulation of those of the zinc wall, so that the temperature of the iron would be quickly brought to that of the copper wall and would vary with it. Experience has shown, however, that in some circumstances the change in thermal conditions of the iron may lag somewhat behind that of the copper wall; hence, it is much more desirable to keep the temperature of the walls of the chamber as constant as possible for the whole length of an experimental period than to depend upon the correction for change in temperature. With a sudden change in the rate of dissipation or absorption of heat in the chamber near the close of a period, which would affect the temperature of the copper wall, there might be an error in the measurement of heat for the period in spite of the allowance for temperature change. (See p. 346.)

CHANGE IN BODY TEMPERATURE OF THE SUBJECT OF AN EXPERIMENT

When the human body is the source of heat in the chamber, allowance must be made for the heat involved in any change in its temperature, as the body has a considerable thermal capacity. From the best available data it would appear that a change of 1 degree in the temperature of the body involves a change of 0.83 Calorie in the quantity of the heat accumulated for each kilogram of body weight. A rise in body temperature would mean that the store of heat in the body has been increased a certain amount, which would have to be added to that eliminated by the body and measured by the calorimeter during the period in which the rise

occurred in computing the quantity of heat actually produced by the body in the period. Conversely, a decrease in body temperature would mean that a certain amount of the heat that had accumulated in the body previous to the experimental period had been eliminated with that produced by the body during the period and should be subtracted from the quantity measured by the calorimeter in determining the quantity actually produced in the period.

The weight of the body can be ascertained accurately. The specific heat assumed is an estimate, but is probably fairly accurate. The temperature of the body as a whole can not be determined precisely, because it is not the same in all parts of the body. The temperature at the surface is noticeably lower than that of the interior, and that of the tissue in one region differs from that of the tissue in another. It seems probable, however, that, except perhaps at the surface, a change in temperature in one part of the body is accompanied by a corresponding change in the others; hence, the amount of temperature change, which is the factor concerned in the correction here considered, may be ascertained with a fair degree of accuracy from measurement of temperature where possible, but preferably below the surface.

By means of an electric-resistance thermometer the temperature of the subject in the chamber, at the spot at which the thermometer is located, may be ascertained at any given moment by the observer outside. A coil of wire of variable resistance, mounted so that it may be worn by the subject and kept at the temperature of the body, is connected with a Wheatstone bridge on the observer's table, by which the variations in resistance of the coil, due to changes in body temperature, may be observed, connection between the bridge and the thermometer coil being made through the special switch mentioned on page 334.

One type of thermometer bulb, designed for use in the rectum, is a coil of platinum wire having a resistance of 20 ohms at 37° C., inclosed in a thin steel shell or capsule 5 cm. in length and 5 mm. in external diameter. Since this thermometer may be kept in place for considerable periods without discomfort, a virtually continuous record of body temperature may be obtained, depending upon the frequency of the readings by the observer, and fluctuations may be followed for long or short periods as desired, but the temperatures at the beginning and end of the experimental period are the ones essential for the correction here considered. In another type, designed for measuring temperature of the body surface, the wire is wound in a flat spiral coil 15 mm. in diameter, mounted in a frame of thin, hard rubber by which it may be held against the skin. This coil rapidly acquires the temperature of the skin.

In some cases the temperature is measured by means of accurate clinical thermometers, inserted by the subject under the tongue or in the axilla, which are afterward read by the observer.

HEAT FROM OTHER SOURCES

The store of heat in other objects in the chamber than the body of the subject—e. g., furniture—is increased by a rise and decreased by a fall in the temperature of the air surrounding them, and allowance must be made for the effect of such change in their condition upon the measurement of the quantity of heat produced in the chamber. The quantity of heat involved is computed from the weight, the specific heat, and the change in the temperature of the objects. The latter factor is not definitely known, however, as no provision is made for actually measuring the temperature of such objects; the assumption being that their change in temperature will be the same as that of the air, which is determined. Where the change occurs slowly, any error involved in such assumption is probably negligible; but this is not true when any considerable change occurs in a short period. This is another reason for keeping the temperature of the air of the chamber as constant as possible.

Allowance must be made also for gain or loss of heat due to the introduction of objects into the chamber at a temperature above or below that of the air. Hot food or drink, for example, admitted through the food aperture would add heat to that produced in the chamber, while cold material would absorb some of the heat produced. The temperature at which any material is admitted is recorded, together with its weight and character, and from these data, with the specific heats of the various articles, the necessary corrections are computed.

The electric fan by which the air of the chamber is agitated and the electric light, when one is used, both generate heat which forms part of that measured by the calorimeter and for which allowance must be made. The quantity of heat produced is computed according to the formula $\frac{EIt}{4.183}$ = small calories at 20° C., E being the voltage and I the amperage of the current in the lamp and the fan, and t the time in seconds during which it was used.

The divisor, 4.183, is the number of international joules (watt seconds) equivalent to one small calorie at 20° C. (10, p. 255). The lamp and fan are connected in such manner that the voltage and amperage of both may be determined at the same time by calibrated measuring instruments on the observer's table, the readings of which are recorded at regular intervals. That the heat may be generated at a uniform rate, the current is taken from a generator which has an automatic regulator to keep the voltage constant within quite narrow limits.

APPARATUS FOR MEASURING MUSCULAR WORK PERFORMED BY THE SUBJECT OF AN EXPERIMENT

For the study of many problems involving the performance of muscular work some method of measuring the amount of work done is requisite. An apparatus (9, p. 48; 8, p. 11) that was devised in connection with the nutrition investigations of the Department has proved very

successful for measuring work done with the muscles of the legs. The principle of the device is that of the electric brake. It is designated a "bicycle ergometer," since it bears some resemblance to a bicycle; in fact, in its construction all of a bicycle except the wheels was used, and the work done in operating it is of the same kind as that involved in propelling a bicycle. In the ergometer, however, the front wheel of the bicycle is replaced by a pedestal and the rear part of the frame is supported by a rack, so that a heavy copper disk, 40.5 cm. in diameter and approximately 6 mm. in thickness, which replaces the rear wheel of the bicycle, will rotate freely on its ball-bearing axis. An electromagnet is attached to the frame, with its poles on opposite sides of the disk, with the inner edge of the rectangular-pole faces coincident with the circumference of the disk, and with the face of each pole 1 mm. from the surface of the side of the disk. When there is no current in the field coils of the magnet, the amount of energy required to cause the disk to rotate between the pole faces is small, being merely that necessary to overcome the friction of the bearings and the resistance of the air against the moving parts; but when there is a current in the coils, with its resulting magnetization, currents are induced in the disk rotating in the magnetic field, which tend to prevent it from rotating. The brake effect depends upon the flux density of the field, which varies with the strength of the magnetizing current. The amount of work done by the subject on the ergometer is therefore easily controlled by adjusting a rheostat in series with the coils of the magnet until the strength of the current is that which will result in the desired resistance to be overcome in causing the disk to rotate. A particular advantage in this apparatus is the constancy and uniformity with which the effect may be reproduced.

The power applied to the pedals when work is done on this ergometer is converted into heat, a small part of it by the friction of the moving parts of the mechanism and the rest by the energy transformations in the disk. The quantity of heat thus produced varies with the intensity of the magnetic field and also with the rate of rotation of the disk. From calibration of the ergometer in the calorimeter the amount of heat produced by each rotation of the disk in the magnetic field was determined for a considerable variety of conditions of velocity of the disk and strength of magnetizing current within the range commonly employed in experiments. By use of curves derived from the data of calibration the heat equivalent of the external muscular work performed by the subject on the bicycle ergometer is computed directly from the total number of rotations of the disk, as shown by an automatic counter, and of strength of current in the field coils, as shown by an accurate ammeter.

The heat produced in the ergometer is measured by means of the calorimeter, together with that eliminated from the body; but since the former can be ascertained, as just explained, it may be subtracted from the total heat measurement, when the amount of heat produced by the subject in performing muscular work is computed.

TESTS OF THE ACCURACY OF THE RESPIRATION CALORIMETER

At frequent intervals the accuracy with which the respiration calorimeter will function is tested by burning ethyl alcohol in the chamber in such manner as to insure complete combustion and measuring with the apparatus the amounts of oxygen consumed and of carbon dioxide, water vapor, and heat produced. Commercial alcohol, pure in quality and containing about 90 per cent of ethyl hydroxid, is satisfactory for the purpose. The actual percentage is ascertained from determination of the specific gravity of the alcohol. The amount of oxygen that would be required to burn 1 gm. of the commercial alcohol, and the amounts of water vapor and carbon dioxide that would result from the combustion, are computed from the chemical equation for the reactions occurring in the combustion of ethyl hydroxid, with allowance for the water present in the sample burned. The heat of combustion of ethyl hydroxid at constant pressure is taken as 7.08 Calories per gram.

The burner used in these experiments was made of two concentric tubes of thin brass 5 cm. in length, the outer tube being 18 mm. in external diameter. At the lower end each tube is soldered to a brass ring, which provides an annular space between them 3 mm. wide, in which is a wick of asbestos; and as the inner tube is open at both ends, there is a center draft for the flame. No products of incomplete combustion have been found in the air of the chamber during a test in which alcohol was burned with this burner.

The burner is soldered to one end of a piece of 4-mm. copper tubing, the other end of which passes through the wall of the chamber to the alcohol supply outside, from which the burner is fed in such manner that the rate of consumption is uniform. Attached to the outer end of the copper tube is an elbow of 4-mm. glass tubing, with a side outlet in the vertical arm to provide for an overflow. The height at which this outlet is set with relation to the top of the burner governs the rate of consumption of the alcohol. The level having been fixed, alcohol is fed into the vertical tube so that some of it will drop regularly from the outlet. The rate of combustion in the chamber is then very constant. The overflow alcohol is caught in a small bottle, which is weighed with the supply flask at the end of each period, the loss in weight of both containers showing the quantity of alcohol burned. The connection between the overflow bottle and the outlet and also that between the supply flask and the feed tube are such that the loss of alcohol by evaporation is negligible.

The results of alcohol check tests of the respiration calorimeter indicate that the determinations made with it are at least sufficiently accurate for investigations of the character in which it is used. This is shown by the data in Table II, which summarizes the results of two tests selected from many equally good.

In November, 1913, in a 3-hour period, 56.3 gm. of commercial alcohol containing 88.32 per cent of ethyl hydroxid were burned to test the

accuracy of the determinations when heat was produced in the chamber at a rate of about 120 calories per hour. For all four factors the quantities determined were slightly larger than those computed from the composition of the alcohol, the discrepancies amounting to 1.5 per cent for oxygen, 0.7 per cent for water, and the same for heat, and 0.2 per cent for carbon dioxid. The respiratory quotient—i. e., the ratio of the volume of carbon dioxid produced to that of oxygen consumed in the combustion of alcohol—is 0.667; in the test the ratio of the values found was 0.658. Similarly, the ratio of the number of Calories of heat produced to the number of grams of carbon dioxid produced is theoretically 3.705, whereas in the test it was 3.725.

TABLE II.—Data obtained in the combustion of alcohol in the respiration calorimeter

Date.	Oxygen.		Water.		Carbon dioxid.		Heat.		Quotients.	
	Found.	Re-quired.	Found.	Re-quired.	Found.	Re-quired.	Found.	Re-quired.	Respiratory (CO ₂ : O ₂).	Thermal (Cal.: CO ₂).
Nov. 21, 1913...	Gm. 105.1	Gm. 103.6	Gm. 65.4	Gm. 64.9	Gm. 95.1	Gm. 94.9	Cal. 354.2	Cal. 351.7	0.658	3.725
Feb. 18, 1915...	139.0	140.7	97.3	98.5	142.5	143.9	535.8	533.3	.669	3.759

In February, 1915, in a 6-hour period, 85.35 gm. of commercial alcohol containing 88.25 per cent of ethyl hydroxid were burned, the rate of production of heat being about 90 Calories per hour. In this test the heat measured by the calorimeter was nearly 0.5 per cent greater than that computed, whereas the quantities of oxygen, water, and carbon dioxid measured were 1 to 1.3 per cent lower than those computed.

Another test made at frequent intervals provides a check on the accuracy of the calorimetric function of the apparatus. Electric energy is converted into heat within the chamber, and the amount produced in a given period, which can be computed very accurately, is compared with that measured by the calorimeter during the period. The electric energy is converted into heat in a coil of resistance wire suspended in the chamber. The amount of energy that is dissipated is computed from the values for the voltage and amperage of the current in the coil, the time in seconds, and the factor for converting watt seconds to small calories at 20° C., as explained on p. 342.

The resistance of the heating coil depends upon the desired heat production, the majority of the tests having been made with a coil having a resistance of 440 ohms, which allows 0.5 ampere of current to flow with a fall of potential of 220 volts. The resultant heat is approximately 95 Calories per hour, or about that produced by an average man sitting still. That the rate of production of heat may be constant, the voltage of the current is controlled by an automatic regulator; but the actual fall in potential is measured by a voltmeter connected to the terminals

of the coil, and the amperage of the current is measured by a milliammeter in series with it, the readings of both meters being recorded every minute, or oftener.

In a 2-hour test in January, 1915, the total heat production was 139.00 Calories, while the quantity measured with the calorimeter was 139.04 Calories. Such absolute agreement is not to be expected invariably, though with the conditions ordinarily prevailing in an electric check the two values should not differ by as much as 1 per cent. A discrepancy of that size would indicate need of attention to some part of the apparatus, or lack of attention to some details of operation. For example, in a 3-hour test in February, 1915, the total quantity of heat generated in the chamber was 370.19 Calories and that measured by the calorimeter was 377.39 Calories. The discrepancy between the two values was due to a considerable decrease in the temperature of the walls of the chamber during the last half of the first hour, resulting from a lowering of the temperature of the water entering the heat absorber. In the two hours following that the heat production was 245.87 Calories, as computed, and 246.75 Calories, as measured, a discrepancy of less than 0.4 per cent. The results in the first hour illustrate the statement made on page 340 regarding error in heat measurement when the temperature of the copper wall changes so quickly that its thermal conditions differ from those of the iron structure to which the wall is attached.

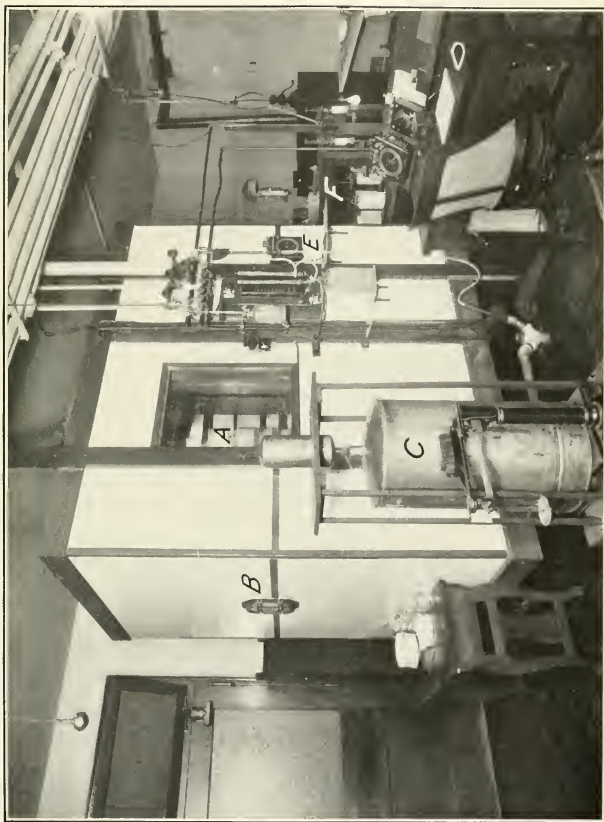
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PLATE XXX

General view of the respiration calorimeter: *A*, Opening serving as door and window to chamber. *B*, Food aperture. *C*, Tank to catch water coming from the heat absorber in the chamber. *D*, Observer's table, with devices for measuring and regulating temperatures. Other temperature measuring and regulating apparatus pertaining to the calorimeter are not shown in this view. *E*, Thin rubber bag, resting on shelf, to serve as air tension equalizer. *F*, Table on which are motor and blower for maintaining circulation of air through chamber, and absorbers for purifying the air.



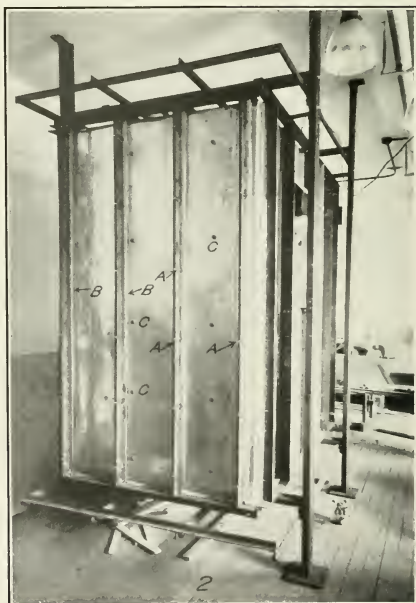
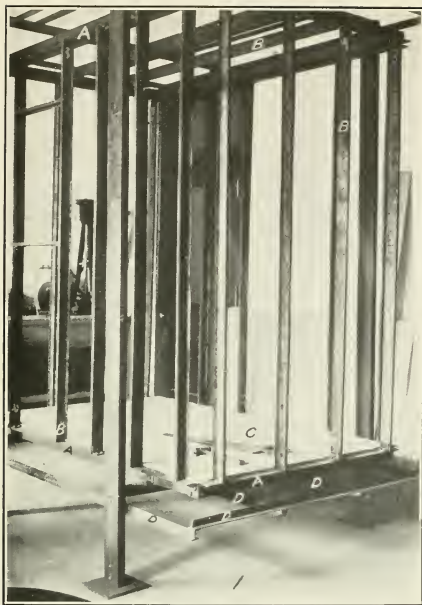


PLATE XXXI

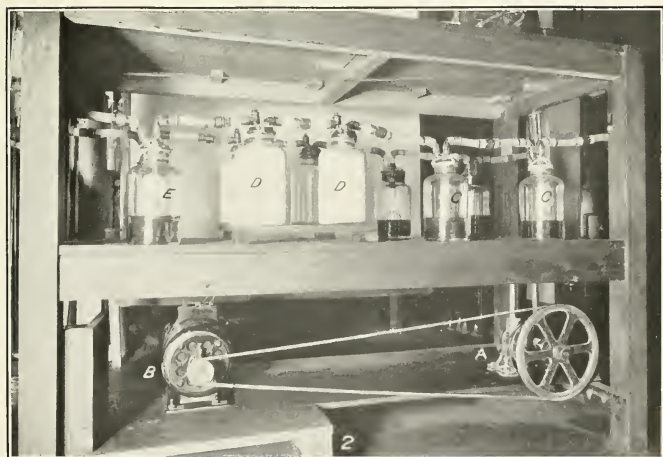
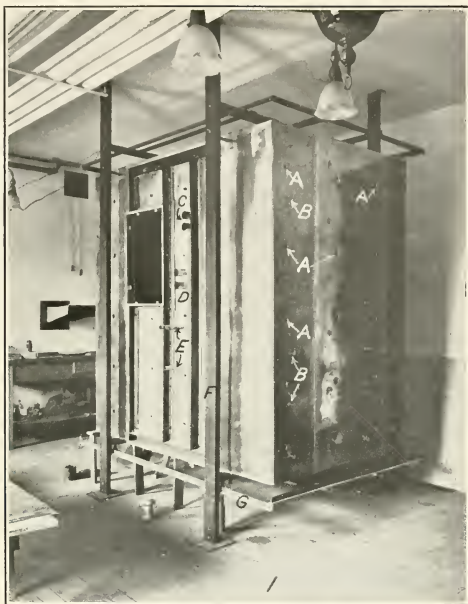
Fig. 1.—Structural iron framework for respiration chamber: *A*, Sills and ceiling plates of angle iron; *B*, studding and joists of light weight channel iron, with narrow edge; *C*, asbestos building lumber to support copper floor; *D*, supports for exterior cover of cork board and museum board.

Fig. 2.—Copper-walled chamber attached to inside of iron framework: *A*, Bolts for attaching wall to structure; *B*, wooden insulation between iron and copper; *C*, thimbles soldered to outside of copper wall to receive inner end of thermoelement described on page 331.

PLATE XXXII

Fig. 1.—Zinc wall attached to outside of iron framework, with all but the last section shown in place: *A*, Openings in zinc wall to admit thermoelements as described on page 332; *B*, hooks projecting outward from edge of channel irons, to support water pipe for cooling zinc, as explained on page 335; *C*, sheet-metal tubes projecting from holes in copper wall for passage of pipes for air entering and leaving the chamber, as explained on page 304; *D*, tubes projecting from holes in copper wall for passage of pipes for water entering and leaving the heat absorber, as explained on page 316; *E*, passages for pipe carrying oxygen into the chamber (see p. 309), for wires for electric fan, electric light, resistance thermometers, etc.; *F*, supporting structure for chamber; *G*, supporting structure for cork board and outer covering.

Fig. 2.—Devices for circulating and purifying air: *A*, Rotary compressor for maintaining air circulation; *B*, motor for driving compressor; *C*, special bottles containing sulphuric acid, for removing water vapor from circulating air; *D*, bottles containing soda lime for absorbing carbon dioxide from circulating air; *E*, bottle containing sulphuric acid for absorbing water vapor given up by the soda lime.



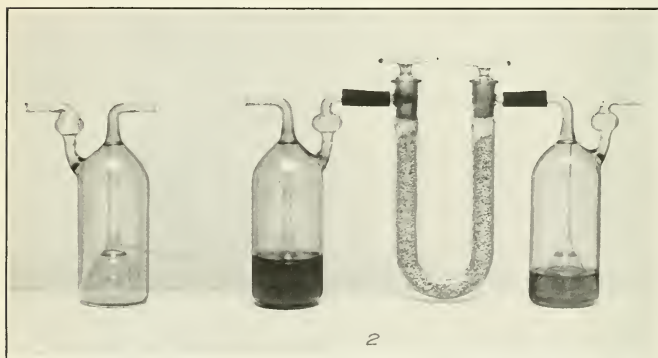
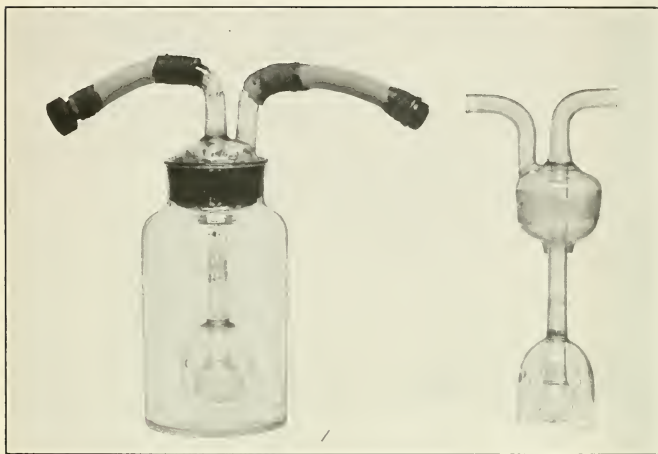


PLATE XXXIII

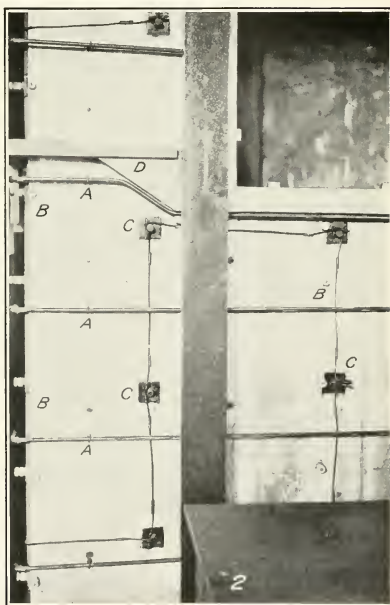
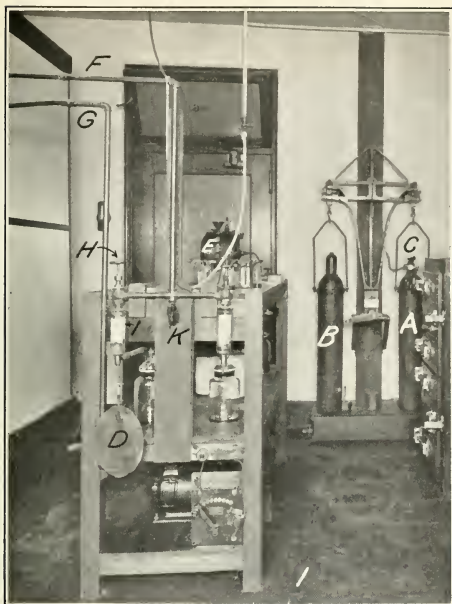
Fig. 1.—Special container for sulphuric acid to remove water vapor from air passing through it. At the right is a stopper with the entrance and exit tubes, as described on p. 366.

Fig. 2.—A small absorber train for removing water vapor and carbon dioxid from sample of residual air. An empty acid bottle is shown at the left.

PLATE XXXIV

Fig. 1.—Balance for weighing oxygen cylinder and end view of absorber table: *A*, Cylinder containing oxygen under pressure; *B*, empty cylinder for counterpoise; *C*, valve for reducing pressure of oxygen from cylinder; *D*, rubber bag to collect sample of air for determination of residual oxygen; *E*, meter for measuring sample of air for determination of residual moisture and carbon dioxide; *F*, pipe for air returning to chamber; *G*, pipe for air coming from chamber; *H*, valve for closing circulating air system between absorbers and ingoing air pipe; *I*, trap for removal of sulphuric-acid spray from returning air; *K*, point at which air from meter is restored to air returning to chamber.

Fig. 2.—Method of attaching heating and cooling systems to zinc wall: *A*, Hooks of iron wire, screwed into metal framework, supporting brass pipe for cooling zinc wall; *B*, porcelain insulators, carrying resistance wire for heating zinc wall; *C*, exterior ends of wooden supports of thermoelements projecting from zinc wall; *D*, wooden strip, dividing air space adjacent to zinc side walls into upper and lower zones.



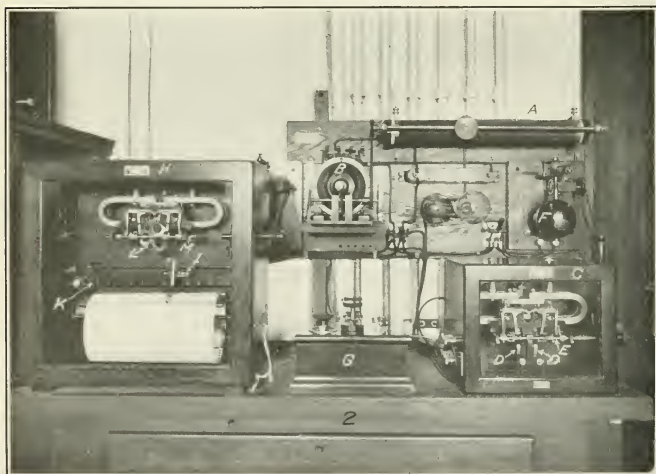


PLATE XXXV

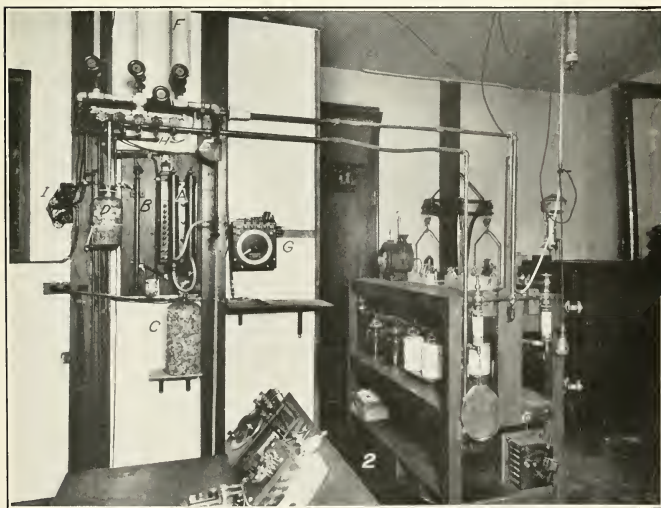
Fig. 1.—Interior of respiration chamber with subject as seen through the window: *A*, Units of the electric-resistance thermometer for determining temperature of the air; *B*, telephone for communication with observer; *C*, push button to call observer; *D*, electric fan to stir the air; *E*, portion of heat-absorbing device described on page 316; *F*, unit of electric-resistance thermometer to measure temperature of copper wall; *G*, small electric lamp for convenience of subject.

Fig. 2.—Apparatus for regulating and measuring the temperature of water for absorbing heat: *A*, Rheostat in series with heating coil in final heater; *B*, differential motor for adjusting sliding contact on rheostat; *C*, mechanism for governing activity of motor *B*, in accordance with deflections of galvanometer mounted in the apparatus; *D*, contact keys for shunting field windings of motor *B*; *E*, shaft turning cams which operate contact keys; *F*, motor driving cam shaft; *G*, special Wheatstone bridge for differential resistance thermometers, containing also devices for varying the range of the slide wire and for checking the precision of the apparatus; *H*, mechanism for shifting balancing contact on slide wire, according to deflections of the galvanometer mounted in the apparatus. The slider *I*, carrying the contact on the slide wire mounted in bar *K*, is moved in either direction by a cord pulled by the shaft bearing large wheel *L*, which is turned by small gears on shaft *M*, driven by a small motor behind the mechanism. A pen carried by the slider draws a temperature difference curve showing the movement of the contact on the slide wire.

PLATE XXXVI

Fig. 1.—Observer's table: *A*, Multiple-point switch for connecting electric measuring circuits with the galvanometer; *B*, Wheatstone bridge (temperature indicator) for measuring temperatures of air in the chamber, of the copper walls, and of the body of the subject; *C*, galvanometer used with the switch and the bridge. The instrument shown here is simply a substitute for a much more sensitive galvanometer which does not appear in this view; *D*, telephone for communication between the observer outside and the subject inside the chamber; *E*, push button to call the subject; *F*, resistance units in series with heating coils outside of zinc wall, as explained on page 336; *G*, rheostats to control currents for heating zinc walls.

Fig. 2.—Devices for regulating temperature of water for heat absorber: *A*, Preheater, adjusted by hand; *B*, final heater, adjusted automatically, having an electric heating coil in the bottom and a resistance thermometer coil in the top of the tube; *C*, bottle filled with pumice, for mixing water flowing from preheater to final heater; *D*, bottle for mixing water flowing from final heater to heat absorber; *E*, special cock to regulate rate of flow of water in heater; *F*, pipe bringing chilled water from cooler to preheater; *G*, temperature indicator connected with thermometer in final heater; *H*, needle valves to regulate flow of water to cool air space adjacent to zinc wall; *I*, exterior ends of electric-resistance thermometers in water entering and leaving heat absorber. Leads from these thermometers extend to the bridge marked "G" in Plate XXXV, figure 2.



OCCURRENCE OF MANGANESE IN WHEAT

By WILLIAM P. HEADDEN,

Chemist, Colorado Agricultural Experiment Station

The presence of manganese in various plants has been observed repeatedly. It is now stated that it has been shown to be present and has been determined in a great many grains, roots, leaves, and whole plants and that it is probably present in all plants.

It is generally asserted that this manganese is to be considered as an accidental constituent and that it has no physiological function. An opposite view, however, is held by some who maintain that it performs an important catalytic function in the plant. Bertrand, for instance (1),¹ has shown that the enzym laccase can not act as an intermediary between the oxygen of the atmosphere and certain organic compounds in the cells of the plants if it is deprived of its manganous oxid, with which it forms a feeble compound. He has further shown that this enzym, laccase, is very generally diffused throughout the vegetable kingdom and that whenever laccase functions in the nutrition and growth of plants, manganese is a necessity. The amount of manganese contained in this laccase is only 0.12 per cent.

There are some data on the effects of manganese on wheat, oats, barley, grass, etc. Guthrie and Cohen (3) attribute the death of grass on certain spots to the presence of 0.254 per cent of manganic oxid in the soil. They account for the death of barley in certain soils in the same manner. Voelcker (7), experimenting with different salts of manganese, applied in quantities up to 200 pounds per acre, obtained results which are summarized as follows for experiments with wheat:

The chlorid and nitrate produced a good color in the plants. The iodid distinctly retarded germination and growth. The untreated plots produced as good plants as any of the others except those which had received an application of nitrate or phosphate. The phosphate, chlorid, sulphate, and red oxid (Mn_3O_4) each gave an increase in yield.

Kelley (6) states that some plants vegetate normally in the presence of manganese salts, others are stunted in growth and die back from the tops of the leaves, which turn yellow or brown and sometimes fall off. Plasmolysis is produced. Chlorophyll may be destroyed as in the pineapple. The ash shows an increased percentage of manganese. The percentage of lime is increased and that of the magnesia is decreased. This ratio, on the authority of Loew, is considered an important one. The absorption of phosphoric acid is lessened. The formation of manganous phosphate is suggested as the possible cause of this.

¹ Reference is made by number to "Literature cited," p. 355.

According to Kelley, the action of manganese, especially if present in the soil in relatively large quantities, 2.34 to 9.74 per cent, produces very radical changes in the nutrition of the plant (5). But such quantities of manganese as correspond to these percentages are not often found in soils.

Brenchley (2, p. 583) sums up her observations on the effects of manganese on barley thus:

Manganese sulfate, though not an actual toxic to barley, retards the growth very considerably if supplied in moderate quantities. Minute traces of the salt have a decided stimulative action both on the root and shoot. * * * When supplied in sufficient concentration manganese is taken up by the plant and deposited in the lower leaves.

Jost (4, p. 87), in treating of the nonessential ash constituents absorbed from the soil by plants, says of silica:

Although silica may be quite superfluous from the chemical point of view, it may be of great service to the plant in the biological sense. Our knowledge of these subjects, despite the amount of work which has been expended on them, is still very imperfect, and it is possible to defend the assertion that all the ash constituents have definite functions to perform, although these have not as yet been determined in all cases, and although these constituents can not be considered as taking part in metabolic changes. * * * The occurrence of manganese may, however, be specially noted, as leading to the consideration of a new series of phenomena. It is not widely distributed in the earth, and yet is found, though only in traces, in very many plants.

In discussing nutritive and stimulative materials he uses the following language concerning iron (4, p. 88):

This distinction is not readily made out in all cases; iron, for example, is a difficult element to deal with, because it is essential only in the minutest traces, and is possibly both a nutrient and a stimulant.

Iron is definitely recognized as essential for the growth of plants, though the quantities required are exceedingly small.

The presence of manganese in wheat straw has been mentioned by others, but nowhere have I found its quantity given, and it is not mentioned in connection with the grain. The statement of M. Bertrand (1) that "manganese has been found in many grains"¹ is the only one known to me that may indicate the occurrence of this element in the wheat kernel.

In examining the mineral constituents of wheat (*Triticum* spp.) I was struck by the fact that there was uniformly enough manganese present to come down with the calcic oxalate and to impart a decided brown color to the calcic oxid when ignited. A few preliminary determinations revealed the fact that there was as much or more manganese than iron present. At the time this observation was made I had examined 25 samples of wheat and had found manganese present in every sample. These samples had been grown on the same soil, though the different plots had not received the same fertilization. The supply of manganese

¹ Author's translation.

in the soil is about 0.10 per cent, calculated as elemental manganese. If the manganese be an accidental constituent, as is usually held to be the case, its presence must be due to the supply in our soil, but the amount taken up appears to be very nearly constant, irrespective of the soil.

In order to ascertain whether the manganese is universally present in the wheat kernel and to determine in what quantity it is usually present, I obtained samples of wheat from a number of localities in the United States and Canada and from three European countries. While manganese is probably present in every cultivated soil, it is very rarely the case that it constitutes more than a small fraction of 1 per cent, while iron is usually present in much more considerable quantities. The amount of manganese present in the soil bears no relation to that of the iron. In the soil on which our wheat samples were grown, the metallic iron found by a mass analysis of the soil was a trifle over 30 times as great as the total amount of manganese. The analytical results given subsequently show that this is not the ratio in which the two elements are present in the kernels and not even in the green plant or in the ripe straw. It does not seem probable that the manganese has been absorbed simply because it existed in the soil associated with iron, if this indeed be the case in any strict sense, for the association might be with calcium as well as with iron.

The method used in determining manganese in grain was to take 10 gm. of ground, air-dried wheat, dissolve it in concentrated nitric acid, and evaporate the solution to a thick, gummy, brown mass. This was then heated over a free flame till all volatile matter was expelled. The dish was then placed in a muffle and most of the carbon burned off. After removal and cooling, a few (4 or 5) cubic centimeters of concentrated sulphuric acid were added. The sides of the dish were washed down with a little water and the solution evaporated at last on a sand bath till vapors of sulphuric acid escaped freely. After cooling, this was taken up with water, boiled, and filtered into a 200 c. c. flask. The residue on the filter was burned, taken up with a little sulphuric acid as before, and the solution filtered and added to the first filtrate. The combined filtrates should be about 150 c. c. in volume and contain about 5 per cent of sulphuric acid. A little silver sulphate (from 25 to 30 mgm.) was next added and then 4 or 5 gm. of ammonic persulphate. The solution was placed on a boiling water bath and allowed to stand as long as the color deepened. It was then cooled, made up to volume, and compared with the standard, which had been prepared in the same way. All reagents should be tested by making a blank.

Manganese in the straw was determined in the same way, except that the silica was removed by evaporating in a platinum dish with the addition of hydrofluoric acid.

In Tables I and II are given the variety, the fertilizer applied, and the percentage of iron and manganese found in wheat from Colorado and

Idaho. Table III gives the percentage of manganese only in wheat from different regions, while Table IV gives the percentage of manganese found in various other grains.

TABLE I.—*Iron and manganese in Colorado wheat*

Variety.	Fertilizer per acre or other treatment.	Iron.	Manganese.
		<i>Per cent.</i>	<i>Per cent.</i>
Defiance.....	80 pounds of nitrogen.....	0.005	0.005
Do.....	40 pounds of phosphorus.....	.005	.004
Do.....	150 pounds of potassium.....	.003	.004
Do.....	None.....	.003	.004
Red Fife.....	80 pounds of nitrogen.....	.004	.004
Do.....	40 pounds of phosphorus.....	.004	.004
Do.....	150 pounds of potassium.....	.004	.005
Do.....	None.....	.005	.005
Kubanka.....	80 pounds of nitrogen.....	.004	.005
Do.....	40 pounds of phosphorus.....	.003	.004
Do.....	150 pounds of potassium.....	.003	.005
Do.....	None.....	.004	.005
Defiance.....	Fallowed 1 year.....	.006	.005
Marquis.....	do.....	.005	.005
Red Fife.....	do.....	.005	.007
Kubanka ^a	do.....	.003	.003
Kubanka ^b	do.....	.004	.004

^a Yellow berry; soft.

^b Flinty; hard.

TABLE II.—*Iron and manganese in Idaho wheat^a*

Variety.	Fertilizer per acre.	Water per acre.	Iron.	Manganese.
		<i>Feet.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Marquis.....	None.....	1	0.006	0.006
Do.....	do.....	2	.005	.006
Do.....	do.....	3	.007	.005
Do.....	16 loads of manure.....	1	.006	.006
Do.....	do.....	2	.006	.005
Do.....	do.....	3	.006	.005

^a I am indebted to Mr. Don. H. Bark, of Boise, for these samples.

TABLE III.—*Manganese in wheat from different localities^a*

Variety.	Locality.	Per cent.	Variety.	Locality.	Per cent.
Poole.....	Missouri.....	0.007	Huron.....	Province of Quebec, Canada.	0.006
Do.....	Ohio.....	.006			
Jones's Long-berry.	Pennsylvania..	.006	Kubanka.....	Petrograd, Russia.	.004
Iowa Red.....	Kansas.....	.005	Bore.....	Svalof, Sweden	.004
Mealy.....	New York.....	.008	Wilhelmina...	Holland.....	.004
Red Fife.....	Province of Manitoba, Canada.	.006			

^a I am under obligation to the respective officers of the various experiment stations for the samples of American and Canadian wheats and to the Bureau of Plant Industry, United States Department of Agriculture, for the samples of foreign wheats.

TABLE IV.—Manganese in other grains

Variety.	Locality.	Per cent.
Emmer (<i>Triticum dicoccum</i>).....	Fort Collins, Colo.....	0.004
Rye (<i>Secale cereale</i>).....	do.....	.004
Barley, bald (<i>Hordeum</i> sp.).....	do.....	.002
Oats (<i>Avena sativa</i>).....	do.....	.005

Several samples of corn (*Zea mays*) were tested, a large white variety (Meerschaum) from Missouri, a yellow variety, irrigated, from Grand Junction, Colo., a yellow variety, not irrigated, from Akron, Colo., and a white variety, irrigated, from Fort Collins, Colo. These samples contained so minute a trace of manganese that it could be detected only with great difficulty when 10 gm. of the grain were used for the test.

In addition to the determinations of manganese given in the preceding tables, I have found it uniformly present in the ash of Colorado wheats and also in wheats from California, Nevada, Washington, Montana, South Dakota, Minnesota, Kentucky, and Tennessee. It can, I believe, be accepted as being universally present in the wheat kernel and likewise in the wheat plant, but it is not as abundant in the dried plant as in the kernels. The ratio of the iron to manganese is higher in the plant. The risk of obtaining iron from dust, etc., in the case of the plant is, it is true, greater than in the case of the kernel, but I think that we are fairly safe in assuming that the iron found in our samples belongs to the plant constituents and is not derived from extraneous sources (Table V).

TABLE V.—Iron and manganese in dried wheat plants

Variety.	Date.	Fertilizer per acre.	Iron.	Manganese.
			Per cent.	Per cent.
Defiance.....	July 28, 1913..	120 pounds of nitrogen.....	0.004
Do.....	do.....	60 pounds of phosphorus....	0.010	.003
Do.....	do.....	200 pounds of potassium....	.010	.003
Red Fife.....	do.....	60 pounds of phosphorus....	.010	.004
Do.....	do.....	200 pounds of potassium....	.015	.004
Kubanka.....	Aug. 6, 1914..	60 pounds of phosphorus....	.008	.002
Do.....	do.....	200 pounds of potassium....	.013	.002

Of the preceding samples only the last two were ripe; the others were cut from 8 to 12 days before being ripe enough to harvest.

The iron present in the straws is from two and one-half to six times as great in amount as the manganese, while in the kernels the manganese is approximately equal to the iron and at the same time is higher, as a rule, than in the straw.

The iron was determined gravimetrically in every case and the manganese colorimetrically. The variation in the iron found is great if calculated on the minimum amount found; still the difference between the

minimum and maximum, in spite of the difficulties of the analysis, is only 0.004 per cent, calculated on the air-dried wheat. The quantity of manganese found shows about the same maximum variation, but the determinations are mostly quite uniform without regard to the State or country in which the wheat was grown.

The samples given represent great differences in cultural conditions of both climate and soil, and yet the manganese is always present and in approximately the same quantities; in fact, a greater regularity is found in this respect than for iron in the determinations made. Iron is accepted as an essential constituent of the plant, while the manganese is held to be a nonessential one by most writers.

Bertrand (1), however, has shown that manganous oxid is essential to the action of laccase; and further, that this enzym is universally present in plants and fulfills a definite function in their metabolism, from which he concludes that manganese is an essential mineral constituent of most, if not of all, plants.

The reaction shown when a fresh surface of a potato is treated with a tincture of guaiacum is attributed to the oxidizing action of laccase. If the statements of Bertrand be correct the potato should contain manganese. For this reason I determined the manganese in a potato, using a single tuber, and found the amount of manganese in this potato, which had been dried at 100° C. for 24 hours, to be 0.0003 per cent, corresponding to from 0.00005 to 0.00006 per cent of the fresh tuber. This quantity seems very small, but even much smaller quantities of manganese in nutritive solutions produce decided effects upon vegetation. Brenchley (2, p. 579), in discussing her experiments to determine the effects of manganese upon the growth of barley, says:

At this date [11 weeks from the beginning of the experiment] it was evident that manganese was deposited in the leaves even at so low a concentration as 1:1,000,000 M. S. and in some cases traces could even be observed in 1:10,000,000 M. S.

The percentages given in my determinations are for elemental manganese; Brenchley used manganous sulphate with five molecules of water. She points out that the effects of manganese may be modified by the relative supply of nutrients.

SUMMARY

(1) Manganese seems to be present in wheat wherever grown, irrespective of the conditions of soil and climate.

(2) Manganese is present in the wheat kernel in about the same proportion as iron, though iron greatly predominates in soils.

(3) Fertilizers applied to the soil did not affect the amount of manganese stored in the kernels.

(4) Variation in the quantity of water applied, from 1 to 3 feet, did not affect the amount of manganese in the grain.

(5) I do not wish to draw conclusions from my facts relative to the essential character of manganese as a mineral constituent of plants, though these facts seem to support this view for wheat and possibly for emmer, rye, oats, etc. It seems improbable that a nonessential constituent would occur in all samples and in essentially the same quantity under such a variety of conditions.

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ASH COMPOSITION OF UPLAND RICE AT VARIOUS STAGES OF GROWTH

By P. L. GILE, *Chemist*, and J. O. CARRERO, *Assistant Chemist*, Porto Rico Agricultural Experiment Station

INTRODUCTION

The following ash analyses of upland rice (*Oryza sativa*) at various stages of growth were made in connection with a study of the effect of lime-induced chlorosis on the ash composition of the plant. In the course of this work it was necessary to know particularly how the iron content of the plant varied with its age. The analyses are reported here, as it is believed that such data are of general importance in explaining certain peculiarities of crop growth.

Kelley and Thompson¹ have already investigated the composition of rice at different stages of growth, but their study did not suffice for our purpose, as it covered only the last half or third of the growing period and did not include iron and some other ash constituents.

EXPERIMENTAL METHODS EMPLOYED

The plants were grown in large cylinders sunk in the ground and protected by wire netting (4 meshes to the inch). Each cylinder afforded a surface of 7 square feet of soil in which 29 plants were grown.

Porto Rican red-clay soil, which is well adapted for rice, was used in the cylinders. This was fertilized liberally with sulphate of ammonia, acid phosphate, and muriate of potash, so that the ash composition might not be influenced at any stage by a lack of nutrients. Fertilizers furnishing 10 gm. of nitrogen (N), 5 gm. of phosphoric acid (P_2O_5) and 10 gm. of potash (K_2O) were incorporated with the soil before planting; when the plants were 18 days old, a surface application of 2 gm. of nitrogen, 1 gm. of phosphoric acid, and 2 gm. of potash was made; and when the plants were 59 days old, 3 gm. of nitrogen, 3 gm. of phosphoric acid, and 3 gm. of potash were applied.

¹ Kelley, W. P., and Thompson, Alice R. A study of the composition of the rice plant. Hawaii Agr. Exp. Sta. Bul. 21, 51 p. 1910.

The plants were watered only a few times, during an occasional dry spell, and made an excellent growth. The growing period was from June 15 to October 16, during which time the weather conditions were fairly uniform, with high temperature and humidity. The average monthly mean temperature ranged from 77.2° in June to 79° F. in October. The monthly precipitations from June to September were, respectively, 10.90, 11.98, 11.67, and 8.22 inches. There were, however, some dry spells of a week or 10 days that apparently affected the plants; note of this is made below.

At 18 days the plants were thinned from 40 to 29 in each cylinder, at which number they were kept during growth. The 11 plants removed from each cylinder at this time served for the 18-day-old sample, while for the 26-day-old sample 6 cylinders were cut; for the 48-day-old sample, 5 cylinders; and for the succeeding samples, 4 cylinders each. As it was impossible to remove the roots completely from the heavy clay soil, the weight of the roots is not recorded. The roots were removed, however, as completely as possible for analysis.

In preparing the samples for analysis each leaf and stalk was washed individually immediately after cutting to guard against loss of mineral matter by leaching. Under such conditions there was probably a certain loss of mineral matter from withered leaves, but no appreciable loss from the green leaves. However, this is practically of little importance, as the conditions of washing, while thorough, were no more severe than those to which the plant would be subjected by rainfall. Even digesting the leaves in cold water for 15 minutes extracted little mineral matter from green leaves. Forty-five gm. of green rice leaves previously washed on the plant were stirred up with 1 liter of distilled water. The water on evaporation yielded a residue of 0.008 gm. of mineral matter, part of which was due to minute leaf hairs broken off in the stirring; 9 gm. of withered leaves soaked for 15 minutes in 500 c. c. of water left a residue of 0.057 gm. of mineral matter.

The analytical methods employed were essentially those of the Association of Official Agricultural Chemists,¹ with a few exceptions. Preparation of the ash was by the optional method, igniting over a very low flame without calcium acetate and leaching when necessary. Iron was determined colorimetrically with potassium thiocyanate, this method being preferable to titration with potassium permanganate for the small amounts present.

ANALYTICAL RESULTS

In Table I are given the data on the weight and composition of a single plant with respect to withered leaves, etc., at each period of sampling. The weights of the plants were, of course, accurately determined, the probable error of the weights and percentages of dry matter merely show-

¹ Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908.

ing the degree of accuracy with which each sample represented, in respect to weight and moisture content, the average of all the plants at each period. In calculating the probable error, one cylinder of 29 plants was taken as a unit. The development of the plants at the different stages was as follows: At 18 days the plants were stooling to some extent; at 73 days they were just about to flower; at 103 days panicles were out, but the seeds were only partially formed; at 123 days seeds were fully formed and ripe.

TABLE I.—Weights of the different parts of the upland rice plant analyzed at various periods

Age of plant.	Dry weight of green stalks and leaves per plant.	Dry weight of withered stalks and leaves per plant.	Dry weight of panicles per plant.	Dry weight of whole plant above ground per plant.	Percentage of dry matter in whole plant aboveground.
Days.	Gm.	Gm.	Gm.	Gm.	
18.....	0.132	0.132	18.7
26.....	.581581 \pm 0.035	20.2 \pm 0.08
48.....	4.38	4.38 \pm 0.25	14.6 \pm 0.05
73.....	11.47	0.95	12.42 \pm 1.20	20.9 \pm 0.10
103.....	21.76	4.53	3.24	29.53 \pm 0.99	25.8 \pm 0.99
123.....	23.34	12.12	35.46 \pm 1.90	36.2 \pm 0.16

It will be noted that the percentage of dry matter in the green plant did not rise until the plant had begun to form seeds. Previous to this time the percentage of dry matter in the plant was somewhat irregular, but tended to remain about 20 per cent. The variations in the moisture content of the first four samples are so many times the probable error of each result that they could not be due to poor sampling. There is little doubt that weather conditions affected the amount of moisture or dry matter in the green plant during the first four stages of growth, while the moisture content of the last two samples was controlled chiefly by the physiological changes in the plant—accumulation of carbohydrates and death of old leaves. This seems evident from the records of rainfall. During the eight days preceding the cutting of each sample the number of days with rain and the total precipitation for the eight days were as follows: Previous to the 18-day-old sample, 5 days with rain, 2.80 inches; previous to the 26-day-old sample, 1 day with 0.90 inch; previous to the 48-day-old sample, 6 days with 4.63 inches; and previous to the 73-day-old sample, 1 day of rain with a precipitation of 0.32 inch. The weather was thus relatively wet, dry, wet, dry; and the percentages of moisture in the green plant were respectively high, low, high, low.

The ash analyses of the various samples are given in Table II. The panicles included the seeds and supporting stems. Withered leaves of the 73- and 103-day-old samples were analyzed separately from the green leaves and stalks, but no such separation was made for the 123-day-

old sample, as all the leaves and straw were partially or completely withered at this period. The 18- to 48-day-old samples had no withered leaves, so that these analyses represent the whole plant aboveground.

TABLE II.—Ash analyses of vegetative parts of the rice plant at various periods

Part of plant.	Age of plant.	Percentage of dry mat- ter in green sam- ple.	Percentages in dry mat- ter of—		Percentages in carbon-free ash of—								
			Carbon-free ash.	Nitrogen (N).	Silica (SiO ₂).	Lime (CaO).	Magnesia (MgO).	Iron (Fe ₂ O ₃).	Potash (K ₂ O).	Soda (Na ₂ O).	Phosphoric acid (P ₂ O ₅).	Sulphuric acid (SO ₃).	Chlorin (Cl).
Green leaves and stalks.....	Days. 18	18.7	17.75	56.88	2.21	3.69	0.75	22.91	1.55	7.94	9.24
Do.....	26	20.2	14.97	4.02	56.34	2.40	4.24	.35	17.28	9.76	6.65	5.81	4.47
Do.....	48	14.6	22.21	1.89	62.56	1.73	3.11	.21	14.66	9.57	4.00	4.41	4.10
Do.....	73	20.1	16.95	1.93	64.82	1.73	3.04	.18	16.88	4.55	3.39	3.89	5.37
Do.....	103	21.5	13.28	1.01	67.71	2.12	3.26	.11	9.54	10.31	4.96	3.61	4.52
Withered leaves and stalks.....	123	28.7	20.23	.62	74.00	1.83	2.27	.32	12.65	4.85	1.80	2.52	3.26
Withered leaves.....	73	37.4	30.12	1.16	83.51	3.12	3.08	.97	6.44	1.31	1.33	1.73	1.69
Do.....	103	61.0	27.60	.41	85.10	3.00	2.84	.72	2.74	2.33	.67	1.20	.80
Panicles immature..	103	48.5	8.86	1.18	78.15	1.17	2.68	.07	6.80	3.20	6.91	3.72	.68
Panicles with ripe seed.....	123	72.8	4.82	1.26	68.93	1.31	4.91	.06	9.74	1.04	14.67	5.60

The percentage of iron in the ash of the green straw and leaves decreased regularly and rapidly with the maturity of the plant, the greatest decrease being from the 18-day-old to the 26-day-old sample.¹ The withered leaves had a relatively high percentage of iron. This may be due to the other samples, consisting of both leaves and stalks, or to the fact that the withered leaves of the 73- and 103-day-old sample were the leaves that appeared first—i. e., those forming a large part of the first samples.

The varying percentages of iron in the ash of the green straw and withered leaves agree with some of the results obtained by Arendt² with oats. He found that the lower leaves of wheat, which must have been withered at the later periods of analysis, contained increasing percentages of iron, which were much greater than the percentages of iron in the ash of the upper leaves.

The lower percentages of potash, phosphoric acid, sulphur, chlorin, and nitrogen in the ash of the withered leaves may be due to translocation of these elements preceding death of the leaves or to loss by leaching after death of the tissue.

In Table III is given the ash composition of the roots and of the whole plant aboveground. The roots for analysis were washed with great care,

¹ These results are in accord with many analyses of green rice straw made previously. Four samples of rice straw from plants grown in four different soils for 25 days contained from 2.76 to 1.98 per cent of iron (Fe₂O₃) in the ash, while samples from a crop grown 84 days had 0.31 to 0.18 per cent, and samples from a 129-day-old crop had but 0.12 to 0.10 per cent of Fe₂O₃ in the ash. (Gile, P. L., and Ageton, C. N. The effect of strongly calcareous soils on the growth and ash composition of certain plants. Porto Rico Agr. Exp. Sta. Bul. 16, p. 31, 1914.)

² Arendt, R. F. E., Untersuchungen über einige Vorgänge bei der Vegetation der Haferpflanze. *In* Landw. Vers. Stat., Bd. 1, p. 31-36. 1859.

but it was impossible to wash them white. The analyses show that the material which could not be washed off was probably finely divided ferric oxid. The percentages of iron found in the ash of the roots ranged from 5.36 to 8.48. This was obviously due to iron contamination from the soil. It was evident, however, that this was a selective contamination chiefly of iron particles, as the ratio of Fe_2O_3 to Al_2O_3 to SiO_2 in the soil was about 1 to 1.5 to 6.¹ Thus, a contamination of the soil as such which would have increased the iron content 6 per cent would have raised the silica 36 per cent and the alumina content 9 per cent. As the high iron content of the root ash is thought to be due to selective contamination from the soil, the results for iron are not reported. The percentages of the other constituents, except possibly silica, could not have been materially affected by soil contamination.

TABLE III.—Ash composition of the roots and of the whole rice plant aboveground

Material analyzed.	Age of material.	Percentages in carbon-free ash of—								
		Silica (SiO ₂).	Lime (CaO).	Magnesia (MgO).	Iron (Fe ₂ O ₃).	Potash (K ₂ O).	Soda (Na ₂ O).	Phosphoric acid (P ₂ O ₅).	Sulphuric acid (SO ₃).	Chlorin (Cl ₂).
Whole plant aboveground.	<i>Days.</i> 18	56.88	2.21	3.69	0.75	22.91	1.75	7.94	9.24
Do.....	26	56.34	2.40	4.24	.35	17.28	9.70	6.65	5.81	4.47
Do.....	48	62.56	1.73	3.11	.21	14.66	9.57	4.00	4.41	4.10
Do.....	73	67.24	1.89	3.07	.28	15.54	4.13	3.10	3.61	4.90
Do.....	103	73.29	2.30	3.09	.28	7.46	7.60	3.87	2.94	3.22
Do.....	123	73.43	1.77	2.57	.29	12.33	4.43	3.21	2.86	2.90
Roots.....	18	42.28	3.82	9.68	22.53	2.10	7.33
Do.....	26	35.62	3.75	8.42	15.46	17.23	8.11	5.48
Do.....	48	46.06	3.01	4.36	21.03	6.32	4.98	8.06	2.30
Do.....	73	60.21	2.84	4.30	15.24	3.74	3.02	6.73	1.92
Do.....	103	61.57	2.76	3.84	10.83	4.42	2.46	6.67	.99
Do.....	123	64.70	4.31	3.05	12.47	1.19	2.63	6.87	1.45

The percentages of iron in the ash of the whole plant aboveground showed but little variation after the sharp drop from the 18- to the 26-day-old sample.

Leaving out of consideration the 123-day-old sample, the composition of which was probably influenced appreciably by the leaching of rain, it can be seen that during the growth of the plant the percentages of lime and magnesia in the ash tended to remain constant, the silica increased, the phosphoric acid and sulphuric acid decreased, the potash, somewhat irregular, tended to decrease, and the soda was irregular. The variations in the percentages of soda are somewhat peculiar, the increase from the 18- to 26-day-old sample being out of all proportion to changes in other constituents. Soda in the ash of the roots, however, increased to an

¹ Iron is much higher in the finer soil separates than in the coarser. (Failyer, G. H., Smith, J. G., and Wade, H. R. The mineral composition of soil particles. U. S. Dept. Agr. Bur. Soils Bul. 54, 36 p. 1908.)

equally great extent from the 18- to 26-day-old sample. Variations in the percentages of potash in the ash of the plant aboveground were for the most part accompanied by similar variations in the ash of the roots. The percentages of soda in the ash seem, as a rule, to fluctuate inversely as the percentages of potash. This is in accord with results showing that soda can to a small extent replace or exercise part of the functions of potash.¹

In the ash of the roots lime, magnesia, phosphoric acid, and chlorin all decreased fairly regularly with the age of the sample.

In Table IV are given the percentages of the ash constituents present in the dry matter of the roots and of the whole plant aboveground.

TABLE IV.—Ash constituents in dry substance of the roots and the whole rice plant aboveground

Material analyzed.	Age of material.	Percentage of dry matter in whole plant above-ground.	Percentages of ash constituents in dry substance of plant.										
			Carbon-free ash.	Silica (SiO ₂).	Lime (CaO).	Magnesia (MgO).	Iron (Fe ₂ O ₃).	Potash (K ₂ O).	Soda (Na ₂ O).	Phosphoric acid (P ₂ O ₅).	Sulphuric acid (SO ₃).	Chlorin (Cl ₂).	Nitrogen (N).
Whole plant above-ground	Days.												
Do.	18	18.7	17.75	10.10	0.39	0.65	0.133	4.07	0.28	1.41	1.64	0.67	4.02
Do.	26	20.2	14.97	8.43	.36	.63	.052	2.59	1.47	1.00	.87	.91	1.89
Do.	48	14.6	22.21	13.89	.38	.69	.048	3.26	2.13	.89	.98	.88	1.87
Do.	73	20.9	17.96	12.07	.34	.55	.051	2.79	.74	.56	.65	.88	1.87
Do.	103	25.8	14.99	10.99	.35	.46	.041	1.12	1.14	.58	.44	.48	.94
Do.	123	36.2	14.96	10.99	.26	.38	.044	1.85	.66	.48	.43	.43	.84
Roots.	18	11.71	4.95	.45	1.13	2.64	.25	.86
Do.	26	9.49	3.38	.36	.80	1.47	1.64	.77	.52	1.48
Do.	48	7.82	3.60	.24	.34	1.64	.49	.39	.63	.18	.95
Do.	73	8.32	5.01	.24	.36	1.27	.31	.25	.56	.16	1.09
Do.	103	8.09	4.98	.22	.3188	.36	.20	.54	.08	.75
Do.	123	5.53	3.58	.24	.1769	.07	.15	.38	.08	.66

In the first four samples the percentages of ash in the dry matter of the plant aboveground varied inversely as the percentages of dry matter in the green plant, and, as noted above, the percentages of dry matter seemed to be lower during the periods of greater precipitation. Thus, with dry weather preceding the sample, the percentage of dry matter in the green plant was high and the percentage of ash low.² An average of several crops of rice grown at different times to eliminate the effect of temporary weather conditions would doubtless show gradually increasing percentages of dry matter in the green plant and gradually decreasing percentages of total ash in the dry matter.

¹ Wagner, Paul. Forschungen auf dem Gebiete der Pflanzenernährung. I. Theil: Die Stickstoffdüngung der Landwirthschaftlichen Kulturpflanzen. p. 231, Berlin, 1892.

Hartwell, B. L., and Pember, F. R. Sodium as a partial substitute for potassium. *In* R. I. Agr. Exp. Sta. 21st Ann. Rpt., 1907-1908, p. 243-247. 1908.

² This is probably owing to the fact that during wet weather the growth of new leaves and tissues is especially active, while in dry weather organic matter is formed more rapidly than mineral matter is absorbed.

On account of the fluctuations in the amount of total ash, it is thought that the percentages of the various ash constituents in the dry matter are less significant than the composition of the ash, which would be unaffected by temporary weather conditions.

The plants were not analyzed at frequent intervals while ripening; nevertheless, the preceding work throws some light on the question of loss of mineral elements at this time. In Table V are given the absolute weights of the ash constituents in one plant at 103 and at 123 days.

TABLE V.—Gain or loss of ash constituents by the rice plant aboveground during last 20 days of growth

Material analyzed.	Age of material.	Weight of ash constituents (in grams) in one whole plant aboveground.										
		Carbon-free ash.	Silica (SiO ₂).	Lime (CaO).	Magnesia (MgO).	Iron (Fe ₂ O ₃).	Potash (K ₂ O).	Soda (Na ₂ O).	Phosphoric acid (P ₂ O ₅).	Sulphuric acid (SO ₃).	Chlorin Cl ₂ .	Nitrogen (N).
Whole plant aboveground.. Do.....	Days.											
	103 123	4.427 5.306	3.245 3.896	0.102 .094	0.137 .137	0.012 .015	0.330 .055	0.337 .235	0.172 .170	0.277 .297	0.130 .152	0.143 .154

It is evident that the aboveground part of the plant lost considerable soda between the last two periods. The roots also must have lost considerable soda, as the percentage of soda in the dry matter of the roots dropped from 0.36 per cent at 103 days to 0.07 per cent at 123 days, while the absolute weight of roots could have increased but little during this interval. The results do not show whether there was any loss of the remaining ash constituents. It is only apparent that, as compared with 103 days, the plant aboveground contained at 123 days the same or a slightly greater quantity of all ash constituents except soda. It is, of course, possible that between 103 and 123 days there might have been an increase followed by a loss of the other ash constituents. The marked loss of soda was more than compensated for by a gain in potash. The increases in the other elements were relatively slight, and the apparent losses of lime and phosphoric acid are without significance when the probable errors of the weights of the plant at the two periods are considered.

DISCUSSION OF RESULTS

It is unnecessary to detail all the changes in ash composition that occurred during the growth of the plant, as these are evident in the tables. In common with similar studies of many other plants the percentages of potash, phosphoric acid, and sulphur in the ash and of nitrogen in the dry matter decreased with the age of the plant, while the silica increased.

The results show that while the iron content of the ash of the whole plant varied but little with the age of the plant, the percentage of iron in

the ash of the green straw and leaves decreased markedly with its age. The withered leaves and straw thus contain a much greater percentage of iron in the ash than the active or live parts of the plants. This would indicate that iron, like silica, is not transported or leached from the dead tissue to the same extent as the other mineral elements.

SUMMARY

Ash analyses of upland rice were made at intervals to show the ash composition of the plant, especially in regard to iron content, from an early stage to complete maturity.

The percentages of potash, phosphoric acid, and sulphur in the ash of the whole plant aboveground decreased with the age of the plant, while silica increased and nitrogen in the dry matter decreased with the age.

As compared with 103 days, when the panicles were just out, the mature plant aboveground at 123 days with the seeds ripe contained an equal amount of lime, magnesia, and phosphoric acid, slightly more iron, sulphur, chlorin, nitrogen, and silica, much less soda, and considerably more potash.

The percentages of iron in the ash of the green leaves and straw decreased regularly and markedly with the age of the plant, while the percentages of iron in the ash of the whole plant aboveground remained fairly constant after the 26-day-old sample.

Previous to flowering, the percentages of dry matter in the green plant and of ash in the dry matter seemed to be influenced by the effect of the weather on the growth of the plant.

VARIETAL RESISTANCE OF PLUMS TO BROWN-ROT

By W. D. VALLEAU,¹

Research Assistant in Fruit-Breeding Investigations, Agricultural Experiment Station
of the University of Minnesota

INTRODUCTION

In the control of plant parasites a great deal of attention has recently been paid to the possibilities of producing resistant plants by breeding. In the plum-breeding plots of the Minnesota Fruit-Breeding Station at Excelsior it is very noticeable that the fruit of certain seedling varieties of plums (*Prunus* spp.) appears to rot much more readily than that of others. The rot is due to attacks of the brown-rot fungus, *Sclerotinia cinerea* (Bon.) Wor. As a knowledge of the factors controlling resistance is necessary for intelligent effort in breeding work, a study of the resistance of plums to the brown-rot fungus was begun in the spring of 1913. The following is a report of the results obtained on the nature of parasitism of the fungus and on varietal resistance of plums to the fungus.

HISTORICAL SUMMARY

TAXONOMIC REVIEW

The life history of the brown-rot fungus has been rather completely worked out, both in this country and in Europe. Woronin (1900)² made a very complete comparative study of *Monilia fructigena* and *M. cinerea*. Two years later Norton (1902) discovered and described the apothecial stage of the American form and referred *M. fructigena* Persoon to *S. fructigena* (Pers.) Schröter. Shortly after this, Aderhold and Ruhland (1905) found and described a perfect stage of *Sclerotinia* spp. on apples, which they concluded to be that of *M. fructigena*. They also found a perfect stage of the apricot brown-rot fungus, *M. laxa*, the *Monilia* stage of which can not be distinguished morphologically from that of *M. cinerea*. A comparison of the perfect stage of the apricot fungus with the perfect stage of the peach fungus of this country, sent to them by Norton, showed differences in ascus and ascospore sizes, and these, with the slight differences which they found in the ability of the two species, *S. cinerea* and *S. laxa*, to infect plum flowers, led them to the conclusion

¹ The work was carried on under direction of the Division of Plant Pathology and Botany, Department of Agriculture, University of Minnesota. The writer wishes to acknowledge indebtedness for suggestions, assistance, and criticism to the following: Dr. E. M. Freeman and Dr. E. C. Stakman, Prof. R. W. Thatcher, of the Division of Chemistry, and Dr. M. J. Dorsey, of the Division of Horticulture, in whose laboratory the work was carried on. The writer also wishes to express his appreciation of the assistance rendered by Mr. Ernest Dorsey in the photomicrographic work and to Dr. C. O. Rosendahl for suggestions and the use of apparatus.

² Bibliographic citations in parentheses refer to "Literature cited," p. 392-395.

that the fungus found on the apricot was a species (*S. laxa*) distinct from that found on plums and cherries (*S. cinerea*). They also concluded that the American species must be *S. cinerea*. A comparison of the ascospores of *S. cinerea* with those of *S. fructigena* brought out the fact that the former always contain from one to many oil globules, while the latter contain none.

Pollock (1909), in a study of the Michigan brown-rot fungus, concluded that it was probably the same species which Norton described, and that, so far as the chlamydospore measurements were concerned, it resembled *S. cinerea* more than *S. fructigena*. Pollock also showed that the microconidia observed by Woronin (1888) on certain other species of *Sclerotinia* and by Humphrey (1891) as appearing on plums which did not produce spore tufts were also produced in abundance when ascospores of the American brown-rot fungus were germinated in distilled water.¹

An important taxonomic fact was brought out by Ewert (1912) when he showed that the *Monilia* spores of *S. fructigena* would not live over the winter, while those of *S. cinerea* would. This difference was not due to the effects of cold, as the spores of *S. fructigena* would stand low temperatures. That the spores of the American form would live over the winter was shown by Arthur (1886), who on May 8 germinated spores taken from mummies of cherries which had hung on the tree all winter. Galloway (1889), in May, 1888, germinated spores taken from mummies collected in July, 1886.²

The perfect stage of the cherry brown-rot fungus in Europe was not found until 1912. Westerdijk (1912) described it at this time and concluded (p. 41), from ascus and ascospore measurements, that "Neben den 3 beschriebenen Obstsclerotinien ist dann also eine spezielle Kirschen-sclerotinie aufzustellen." The asci and ascospore measurements presented by Reade (1908) and Pollock (1909), however, do not warrant this conclusion.

Matheny (1913) made an extensive study of the brown-rot fungus from various parts of this country and compared it closely with pure cultures of *S. fructigena* and *S. cinerea* sent to him from Europe. He concluded that the *Monilia* stage in this country agreed very closely with that of *S. cinerea* of Europe and that the apothecial stage differed in shape of spore and in the presence of oil globules in the ascospores from that of *S. fructigena* and referred the American brown-rot fungus to *S. cinerea*. Conel (1914) made a study of the brown-rot in the vicinity of Champaign and Urbana, Ill., and decided, both because of its morphological characters and from the fact that the *Monilia* form is capable of living over winter, that the fungus was *S. cinerea*.

¹ Jehle in an unpublished thesis on file at the University of Minnesota also observed the production of these conidia from ascospores, and on the same hypha observed the *Monilia* spores, thereby definitely connecting the perfect and the *Monilia* stages.

² Jehle also germinated spores found on mummies in the early spring.

PHYSIOLOGICAL REVIEW

A considerable amount of literature has appeared, especially in recent years, on the subject of resistance and immunity to disease. The cereal crops have perhaps received the most attention. Bolley (1889) and Anderson (1890) attempted to correlate resistance with certain morphological characters. Cobb (1892, p. 181-212) advanced the theory of mechanical resistance due to morphological characters, such as thick cuticle, waxy coating, and small stomata. Freeman (1911) showed that barley might escape rust owing to variation in amount of bloom produced on the leaves, which could be varied by growing in soils of different degrees of alkalinity. This escape from rust is not true resistance, but is due to the inability of the water to wet the surface of the leaves so that the drops containing the spores roll off. When these plants were infected, however, they "exhibited large and vigorous growths of the rust."

Marryat (1907) showed in the case of *Puccinia glumarum* grown on a semi-immune host that it killed small areas of the host tissue and formed only small or abortive pustules, while in the case of the susceptible forms the host cells, though containing haustoria, were apparently normal.

Comes (1912) reported that Rieti wheat, which is very resistant to rust, contained a higher percentage of acid than other more susceptible forms and also that the acid content increases with the altitude at which wheats are grown, as does also the ability to resist rust.

Jones (1905) showed that some varieties of potatoes are much more resistant to certain potato diseases than others. He based resistance more on chemical composition than on morphological differences in the host.

Kinney (1897) noted that "fruit of different varieties of plums varies in susceptibility to injury by rot fungus" and attributed the difference in resistance to variations in texture of the skin. He also stated that early varieties are usually injured more than those which ripen their fruit later.

Müller-Thurgau (1900) noticed that varieties of apples in Switzerland showed different degrees of susceptibility to a wilt or blight caused by *M. fructigena*.

Quaintance (1900) observed a marked variation among varieties of drupaceous fruits in their resistance to attacks of the brown-rot fungus. Among the peaches the varieties densely covered with down were the most susceptible. Of the plums some varieties of the Miner group were practically free, those of the Wild Goose rotted about 10 per cent, while the varieties of *Prunus americana*, *P. triflora*, and *P. pumila* were very susceptible. He suggested that the firmness and thickness of the skin of the Miner plums might have something to do with their resistance. The relative resistance of some varieties of *P. domestica* to brown-rot is given by Alwood and Price (1903).

Köck (1910) ascribes the resistance of certain varieties of cherries to a blossom-blight caused by *S. cinerea* to the blossoming of these varieties when conditions are unfavorable for the disease.

Cook and Taubenhaus (1911 and 1912) pointed out the toxic properties of tannins and fruit acids and also showed a relationship to exist between the decrease in oxidizing enzym content of fruits and the increase in their susceptibility to disease.

With regard to the physiological relationship between host and parasite, considerable work has been done. Jones (1910) gave a comprehensive review of the literature on this subject, dealing especially with the bacteria. Cooley (1914) reviewed much of the work on the physiological relations of the fungi. Therefore, only a short review will be given of the literature dealing with *Sclerotinia* spp.

Behrens (1898) in his work on the physiology of *Oidium* (*Sclerotinia*) *fructigenum*, *Penicillium* spp., and some other fungi, concluded that *S. fructigenum* was exclusively an intercellular fungus and did not secrete a cellulose-dissolving enzym. He considered that the fungus split the middle lamella by mechanical force. *Penicillium* spp., he concluded, also did not enter the cells, but did produce a middle-lamella-splitting enzym.

Schellenberg (1908) studied the effect of *S. fructigena* and *S. cinerea* on a number of tissues, but not on their respective hosts. He considered both of these fungi to be intercellular, producing no cellulose-splitting enzym. He thought, however, that they did produce a hemicellulose-dissolving enzym and that the cell walls in contact with the hyphæ were slightly dissolved. He saw no evidences of a middle-lamella-splitting enzym.

Bruschi (1912) noticed, when *M. cinerea* was grown in a medium containing plum flesh, that after 48 hours the cells were all separated from one another, and concluded that the fungus produced the middle-lamella-splitting enzym pectinase. Attempts to isolate a cellulose-dissolving enzym were unsuccessful.

Cooley (1914) demonstrated the ability of *S. cinerea* to produce an enzym which would coagulate pectin from solution in the absence of calcium. This enzym he called "pectinase." His use of this term is, however, not clear, as he states (p. 314) that he adopted "the nomenclature used by Jones and Euler, namely, employing pectinase as the term to designate the enzyme inducing coagulation of a pectin solution and also the hydrolysis of calcium pectate, or pectinate." Jones (1910) used, in a general way, the nomenclature suggested by Bourquelot and Hérissé (1898) regarding the enzym which they extracted from barley malt; as he says (p. 355), "All things considered, we favor the name *pectinase*, which was suggested by Bourquelot and Hérissé, as already explained." On the other hand, Euler-Chelpin (1912, p. 32) states that "The enzyme

here termed pectase was obtained from malt-extract by Bourquelot and Hérissé, who called it pectinase; according to the general principle of naming enzymes after the substrate, this should be altered to pectase." In a subsequent paragraph he states that "By the term pectinase should be indicated the enzyme which coagulates dissolved pectin substances, e. g., in fruit juices, in the presence of lime to gelatinous calcium salts of the feebly acid pectinic acids." If we follow the definition of a pectinase given by Jones and the classification given by Haas and Hill (1913, p. 339), we must refer to the enzyme demonstrated by Cooley as "pectase."

The attempts of Cooley (1914) to isolate a middle-lamella-splitting enzyme from rotted fruit gave negative results. In certain artificial media a cellulose-dissolving enzyme was produced, but its action on cellulose isolated from plums was very slight. From direct observations on the fungus in free-hand sections of fruit he concluded that "the fungus does not show any particular affinity for the middle-lamella, but penetrates and permeates with equal avidity any part of the host tissue." He could find no relationship to exist between varying acid content of plums at different periods of development and increased susceptibility of ripe over green fruits.

EXPERIMENTAL MATERIAL

The organism used in this work was isolated when needed from rotting plums, as it seemed better to use only strains which had been growing under normal conditions rather than to risk a decrease in virulence of infection due to growing a single strain on artificial media.

The plums used consisted for the most part of hybrids produced at the Minnesota Fruit-Breeding Station at Excelsior. Those referred to in the text as "B × W" are hybrids of Burbank (*P. triflora*), the female parent, with Wolf (*P. americana mollis*). The A × W crosses are Abundance (*P. triflora*) × Wolf. The Burbank is a medium thick-skinned variety which becomes soft when ripe and is rather susceptible to the brown-rot. Wolf has a thick, tough skin and is not affected to any great extent by the rot in the field. Abundance is reported by Hedrick (1911) as being less subject to attacks of the brown-rot than Burbank. The crosses B × W15 and A × W18 are both characterized by being very firm when ripe, and are both nearly immune to brown-rot in the field. The other hybrids of these two series vary in firmness and resistance.

Etopa and Sapa (*Prunus besseyi* × Sultan, *P. triflora*) and Wakapa (Red June, *P. triflora*, × DeSota, *P. americana*, but resembling very closely a sand-cherry hybrid) are products of the South Dakota Experiment Station. They are thin-skinned varieties and are susceptible to rot. The sand cherry (*P. besseyi*) is a small fruit which becomes soft on ripening. It has very astringent flesh and is susceptible to brown-rot.

Gold is a thin-skinned susceptible variety. Sultan is not known to the writer.

The three varieties designated "S. D. Nos. 1, 2, and 3" are varieties obtained from Mr. A. Brackett, of Excelsior, who received them from the South Dakota Experiment Station. Their true names were not known to Mr. Brackett. S. D. No. 1 is a thin-skinned variety and rotted badly on the trees when sprayed once with Bordeaux mixture. S. D. Nos. 2 and 3 were thicker skinned, firmer varieties and did not rot after one spraying, many fruits drying on the trees. All appear to be sand-cherry hybrids.

Compass, a hybrid between a sand cherry and *P. americana* (Hansen, 1911), is a thin-skinned variety which becomes soft on ripening and is susceptible to the brown-rot. Reagan, a hybrid of Wayland (*P. hortulana*) \times *P. americana* (Hedrick, 1911) is thick-skinned, very firm when ripe, and is very resistant to the rot. Specimens of the ripe fruit used were received from the New York Experiment Station, Geneva, N. Y.

Ocheeda and Harrison are varieties of *P. americana*. Manitoba No. 1 is probably a variety of *P. nigra*. Hammer is a hybrid between *P. hortulana mineri* and *P. americana* (Hedrick, 1911). These varieties were obtained from the orchard at University Farm.

TAXONOMY OF THE FUNGUS

MONILIA STAGE

The brown-rot fungus in Minnesota is found for the most part affecting plums, but to a very limited extent also attacking the apple. It appears on the plum first as a small brown or purple spot, which increases very rapidly in size. In a very short time the spore tufts appear irregularly over the surface of the rotted area. These are usually small and ashen gray in color, although in many cases the color varies to a yellow ocher. Plums inoculated through a wound made by cutting off the tip of the fruit, when allowed to rot under a cardboard box in nearly total darkness, produced spores of a bright-ocher color over the wounded area and in some cases through the skin. Mummies collected from trees in the late fall showed spore tufts which varied from gray to a light ocher. The chlamydospores of the local form, taken from mummies which have hung on the trees over winter, retain their power of germination.

Chlamydospore measurements were made of spores from Soulard and Longfield apples, from Harrison, Ocheeda, Newman, and Surprise plums, which were rotted in the laboratory, and from a culture on beerwort agar. In each instance 100 spores were measured, except in the case of the beerwort-agar culture, where 50 spores were measured. The results are given in Table I.

TABLE I.—*Chlamydospore measurements of Sclerotinia cinerea*

Medium.	Average length.	Average breadth.	Medium.	Average length.	Average breadth.
Surprise plum.....	μ 16.22	μ 11.24	Longfield apple.....	μ 15.80	μ 10.81
Newman plum.....	17.38	12.10	Soulard apple.....	15.30	10.76
Ocheeda plum.....	16.18	11.09	Beerwort agar.....	14.05	8.77
Harrison plum.....	15.95	10.98			

From a comparison of these measurements with those given in Table II, it will be seen that they agree very closely with those obtained by other investigators in this country and are only slightly larger than those given for *S. cinerea* by European investigators. They also correspond closely to the measurements given by Aderhold and Ruhland for *S. laxa* found on apricots.

TABLE II.—*Spore and ascus measurements of the brown-rot fungus as given by various investigators*

FROM EUROPEAN SOURCES

Fungus and investigator.	Host.	Chlamydospores.	Asci.	Ascospores.
<i>Sclerotinia cinerea:</i>		μ	μ	μ
Saccardo (1886)...		15 to 17 by 10 to 12..		
		12.1 by 8.8 to 13.2		
		by 9.9		
Woronin (1900)...	In culture..	17.5 to 24.2 by 11.2		
		to 13.2		
Aderhold and	Cherry....	13.8 by 9.2.....		
Ruhland (1905).	Various....	13.8 by 9.95.....		
Matheny (1913)...	Peach and plum	14.4 by 10.8.....		
<i>Sclerotinia laxa:</i>				
Aderhold and	Apricot....	16.1 by 10.8.....	121.5 to	11.5 to 13.5
Ruhland (1905).			149.9 by	by 5.2 to
			8.5 to 11.8	6.9
Cherry brown-rot:				
Westerdijk (1912)...	Cherry....		158.4 to	13.2 to 16.8
			171.6 by	by 4.3 to
			7.9 to 8.5	5.2
<i>Sclerotinia fructigena:</i>				
Saccardo (1886)...		25 by 10 to 12.....		
	Apple.....	20.9 by 12.4 to 24.5		
Woronin (1900)...	In culture..	by 13.2		
		23.7 to 30.8 by 14.9		
		to 16.5		
Aderhold and	Apple.....	25 by 13.....	120 to 180	11 to 12.5 by
Ruhland (1905).			by 9 to 12	5.6 to 6.8
Matheny (1913)...		22.1 by 11.2.....		

TABLE II.—*Spore and ascus measurements of the brown-rot fungus as given by various investigators—Continued*

FROM AMERICAN SOURCES

Fungus and investigator.	Host.	Chlamydospores.	Asci.	Ascospores.
<i>Sclerotinia fructigena</i> : Norton (1902).....		"	μ 45 to 60 by 3 to 4	μ
Aderhold and Ruhl- and (1905).....			89.3 to 107.6 by 5.9 to 6.8	6.2 to 9.3 by 3.1 to 4.6
Reade (1908).....		17 by 11.....	125 to 215 by 7 to 10	10 to 15 by 5 to 8
Pollock (1909).....	Plum.....	14.4 to 24 by 9.6 to 14.4	130 to 179 by 9.2 to 11.5	11.4 to 14.4 by 5 to 7
	In culture.....	9.6 to 14.4 by 7.2 to 10.8
Matheny (1913).....	Peach.....	14.7 by 9.9.....	135 to 190 by 6.9 to 10.5	10.5 to 14.5 by 5.2 to 7.5
	Plum.....	135 to 173 by 6.8 to 10.8	9.3 to 14.2 by 5 to 7.4

SCLEROTINIA STAGE

The apothecial stage of the local brown-rot fungus has been found in abundance in the University of Minnesota Experiment Station orchard during the last few springs. It appears during the blooming period of the plums. The ascospores showed the characteristic refractive globules which Aderhold and Ruhland (1905) pointed out as being one of the characters which make it possible to distinguish between *S. cinerea* and *S. fructigena*, the latter species containing none.

Some doubt has existed in regard to the exact time required for the production of the perfect stage after the formation of the sclerotium or mummy. The field observations of Norton (1902) and others seem to indicate that the apothecia are formed the second spring after the rotting of the fruit—i. e., in approximately 18 months. Other investigators (Dandeno, 1908) have thought that they may be produced the spring following the rotting of the fruit. No experimental evidence has come to the notice of the writer which shows definitely the period required for the production of apothecia; therefore, the following experiment was performed.

During the fall of 1913 two lots of mummied plums and one of apples were buried. Lot 1 consisted of 1 plum each of 16 varieties which had been rotted in the laboratory. These were buried on October 8, 1913, about $\frac{1}{2}$ to 1 inch deep in a shallow box, which was then placed level with the ground on a shaded hillside. Lot 2 consisted of (A) 106 fruits from 8 varieties of plums which had rotted in the field under field conditions during the fall of 1913, and (B) 30 mummies of 3 other varieties which

had been hanging on the trees since the fall of 1912. The plums of this lot were buried on October 15, 1913, near the previous lot and when finally examined were buried from $\frac{1}{4}$ to 1 inch deep. The fruit of each variety was kept separate. Lot 3 was made up of 48 apples representing 7 varieties. The fruits had been inoculated through wounds in the laboratory and on October 18, 1913, when entirely rotted, were buried.

The results obtained were as follows: In the spring of 1914 no apothecia were found on any of the three lots. An examination of lot 1 on May 7, 1915, showed 4 of the total of 16 fruits producing a total of 71 cups. On further examination these were all found to be growing from the upper side of the sclerotium. Two others, which had been buried deeper, were found to be producing many of the young cups which at this time had not reached the surface of the ground.

Lot 3 at this time showed no apothecia. On May 12, 1915, lot 2 was examined; of the total of 106 mummies produced in 1913, 39 were producing apothecia in abundance. In a number of other instances the sclerotium was present, but was producing no apothecia. Of the 30 mummies produced in 1912, 4, of the Opata variety, were producing a total of 10 cups, while the sclerotia of the Compass and Topa varieties had entirely rotted. At this time lot 3 was also examined, and as no apothecia were being produced an attempt was made to find the sclerotia. Small pieces of the black, leather-like sclerotia were found where 4 of the varieties had been buried, but in all other cases they had entirely rotted. The sclerotium of a Shields crab-apple had a growth of about one-fourth of an inch upon it which appeared very much like that of a young cup, but when this piece was again buried it showed no further development.

From this experiment we may conclude that for the production of the perfect stage of *S. cinerea* the mummies must be buried for at least two winters and that mummies which have hung on the tree for one year still have the power of producing apothecia.

From a horticultural standpoint it is of interest to note that of the 156 plum pits buried in 1913 none germinated in the spring of 1914, but in the following spring 106 produced young plants. Of these, 6 were of the Topa variety which had hung on the tree for one year before burying.

Measurements were made of asci and ascospores from material collected on April 10, 1914. The asci varied in length from 102 to 166 μ , and in breadth from 3.5 to 5.7 μ . The ascospores varied from 5.6 to 8.9 μ in length and from 2.9 to 3.8 μ in breadth.

Reference to Table II shows the wide range in ascus and ascospore measurements as determined by various investigators, the asci of Norton ranging from 45 to 60 by 3 to 4 μ ; of Aderhold and Ruhland (who received their material from Norton), 89.3 to 107.6 by 5.9 to 6.8 μ , those from the Minnesota Experiment Station, 102 to 166 by 3.5 to 5.7 μ , while the upper extreme is reached by Reade (who also obtained his material from Norton), who found the asci ranging from 125 to 215 by 7 to 10 μ .

By comparing the figures given by Westerdijk (1912) for the cherry fungus with those given above, it will be seen that they fall well within the range of *S. cinerea*, and as this difference in the size of the asci and of the ascospores was the only one upon which she based her conclusion as to its being a separate form, it seems safe to conclude that what she described was the perfect stage of *S. cinerea*.

It has already been pointed out that the Monilia stage of the apricot fungus, described by Aderhold and Ruhland (1905), compares favorably with the Monilia stage of the American brown-rot fungus, and they showed that it was identical, except for slight differences in chlamydospore size, with that of the European *S. cinerea*. By referring to Table II it will be seen that the ascus and ascospore measurements given for the perfect stage of *S. laxa* fall well within the limits determined for *S. cinerea*. Considering the fact that at present there are no known morphological differences between *S. cinerea* and the apricot fungus, is the fact that Aderhold and Ruhland were able to get infection of plum flowers in only a few cases with chlamydospores of *S. laxa* sufficient evidence to make this a separate species?

MICROCONIDIAL STAGE

The microconidial stage, as was stated above, has been described by Woronin for a number of species of *Sclerotinia*, including *S. fructigena* and *S. cinerea*. He, however, could show no differences between the spores of the two latter species, and they are therefore of little value in identification of the species.

The production of the microconidia was first seen by the writer in a potato-plug culture of the local fungus nearly a year old. The spores ranged from 2.2 to 2.6 μ in diameter, were spherical, and contained a large refractive globule. They were later found on agar cultures in great abundance, in hanging drops of distilled water, and also in hanging drops of 1 per cent malic, 0.062 gallic, 0.062 and 0.25 per cent tannic acids. In the latter cases the flask-shaped sterigmata could be seen. Chains of from 15 to 20 spores were not uncommon. They were also produced in great abundance on the surface of a very young Surprise plum picked and inoculated June 3. These spores ranged in size from 2.55 μ to 3.22 μ , averaging for 25 measurements 2.72 μ . The microconidia produced in the 1 per cent malic-acid solution were larger, ranging from 2.60 to 3.79 μ , measurements of 25 spores averaging 3.14 μ .

PHYSIOLOGICAL AND PATHOLOGICAL RELATIONS

INFECTION

Opinions differ as to the ability of the brown-rot fungus to penetrate the uninjured surface of fruits. Peck (1881) was unable to get infection of fruits when the spores were planted on the uninjured surface. Smith

(1889), however, had no trouble in bringing about infection in ripe peaches when he sowed the spores in a drop of water on the uninjured skin. Cordley (1899) obtained similar results with plums and cherries.

Field observations indicate that infection of green plums may take place through the uninjured surface if conditions are very favorable. These cases are comparatively rare, the greatest number of infections in green fruit taking place through curculio or other wounds. It is not rare, however, to find in a rotting condition uninjured green plums which are in contact with a rotting plum that is producing spores. In the ripe fruit it is not at all uncommon to find rot due to infection through uninjured cuticle which is not in contact with that of other plums.

Cooley (1914, p. 322-323) concluded from infection experiments that "The brown-rot organism will infect fruits which are immature, even penetrating those which are not more than half-grown or those in which the pits are still soft, provided the skin is punctured." He had no trouble in infecting ripe fruits without injuring them.

In the following infection experiments, carried on to determine the relative resistance of varieties, results were obtained which differ somewhat from those of Cooley.

On June 14, 1913, five plums of each of seven varieties were put into a sterile chamber and sprayed with distilled water containing *Monilia* spores. The results are set forth in Table III.

TABLE III.—*Results of inoculation of green plums with Sclerotinia cinerea through uninjured cuticle*

Variety.	June 14.	June 16.	June 17.
Etapa.....	Plums inoculated...	1 infection spot....	5 infection spots on 2 plums.
Opata.....	do.....	5 fruits rotting.
Topa.....	do.....	10 infection spots...	3 fruits completely rotted; 2 have 1 spot each.
A × W 15...	do.....	15 infection spots...	Spots spreading slowly.
B × W 21...	do.....	No infection spots...	2 clean; 3 one spot each; not spreading rapidly.
B × W 15...	do.....	do.....	No infection spots.
Americana seedling No. 1.	do.....	1 through curculio wound.	4 clean; 1 completely rotted through curculio wound.

These results show very clearly that infection can take place through the injured skin of very young plums. This experiment was repeated from time to time until the plums were ripe, and at no time, if the temperature was favorable, was any difficulty encountered in obtaining infection through the uninjured surface of certain varieties.

The results given in Table III also indicate that there is considerable difference in the ease with which the varieties of plums are infected, as well as the rapidity with which the fruit rots after infection has taken

place. Is the difference in susceptibility to infection due to differences in morphological characters of the epidermis?

It has been definitely proved from time to time that the fungus has the ability to "bore" through the uninjured skin of plums and peaches. Therefore, penetration must take place either through the rather thick cuticle of the epidermal layer or through the stomata.

MORPHOLOGY OF THE SKIN AND FLESH OF THE PLUM

For a better understanding of the entrance and penetration of the fungus in the plum fruit, a knowledge of the morphology of the "skin" and underlying layers of cells is necessary.

STOMATA.—The epidermis of the plum consists of a single layer of cells covered by a rather thick layer of a cutinized substance (Pl. XXXVIII, fig. 2). On the surface of this is secreted a waxy "bloom."

Stomata are present in the young fruit. In fruit about half grown changes take place in the stomata leading to the formation of lenticels.

The lenticels are formed in at least three ways:

(A) In some cases a few flat disk-shaped cells are formed parallel to the epidermis and lining the stomatal cavity. The walls of these cells appear to be of the same material as those of the deeper lying parenchyma cells (Pl. XXXVII, fig. 1). The guard cells often open wide and dry out. In other cases changes take place in the composition of the walls of about two layers of cells lining the stomatal cavity. These cells, the walls of which were originally cellulose, give the characteristic yellow staining reaction of cork with the iron-alum-hematoxylin safranin stain (Pl. XXXVII, fig. 3).

(B) In some varieties meristematic tissue develops from the parenchyma cells and produces tissue which partially (Pl. XXXVII, fig. 2) or completely fills the stomatal cavity (Pl. XXXVII, fig. 4). Occasionally a column of cells even grows out through the stomatal opening. These cells appear to be of the same nature as the hypodermal cells underlying the epidermis, in no case giving the staining reaction of cork.

(C) The lenticels, which appear as large, corky specks on the surface of ripe plums, are made of a pad of corky cells lying parallel to the epidermis. They probably develop at the stomata, splitting the guard cells apart and growing out through the opening. The details of their formation, however, have not been carefully studied in this connection, as only very few were encountered in the material examined.

HYPODERMAL PARENCHYMA.—Directly underlying the epidermis are layers of oblong cells slightly larger than and lying parallel to the epidermal layer. These make up what is commonly known as the "skin" of the plum. In some of the thick-skinned varieties there are often as many as seven or eight layers of these cells (Pl. XXXVIII, fig. 5), while in the thin-skinned forms often not more than one or two layers are present (Pl. XXXVII, fig. 1, 2, and 5).

Lying below the hypodermal layers of cells and in sharp contrast to them are the large, isodiametric cells which make up the mass of the fruit tissue (Pl. XXXVII, fig. 6). In the ripening process in those varieties which become soft these cells split apart at the middle lamella (Pl. XXXVII, fig. 5). The solution of the middle lamella apparently takes place more readily in these cells than in those of the hypodermal layers.

METHOD OF ENTRANCE OF THE FUNGUS

Two methods were used in the determination of the details of the entrance of the fungus. The first consisted of macroscopic observations on ripe or nearly ripe fruit shortly after infection had taken place. In the second method fruits of a number of varieties of plums at various stages of development were brought into the laboratory and inoculated, in some cases by a suspension of spores in water and in others by laying the plums in contact with moist mummies well covered with spores. After infection had taken place and small decayed spots had appeared, blocks of the flesh, including these spots, were killed and embedded in paraffin, according to the usual methods employed. These were later sectioned, mounted, and stained. Sections 8 to 11 μ thick were found most satisfactory. Various stains were used, including Harper's short modification of the triple stain, Heidenhain's iron-alum-hematoxylin, and also a modification of this in which safranin was used. This last-named stain proved very satisfactory.

It was noticed continually, particularly in ripe or nearly ripe fruit, that when infection took place through the uninjured skin, the spot always had in its center a lenticel or "dot." These observations indicated that infection takes place, not through the cuticle, but through the lenticel in ripe or nearly ripe fruit. Further evidence was obtained on this point when sections were made of the skin from material in which the lenticels were either forming or completely formed and through which infection had taken place. It was found that the hyphæ entered between the guard cells into the stomatal cavity (Pl. XXXVIII, fig. 3, 4, and 5). In those stomata lined with corky material infection of the fruit tissue does not take place immediately, as the fungus apparently has not the power to pierce directly through the corky cells. The hyphæ continue to grow, filling up the stomatal cavity, and eventually exert enough pressure to split away the epidermis from the lenticel cells (Pl. XXXVIII, fig. 5). It is through this opening that infection takes place into the fruit tissue (Pl. XXXVIII, fig. 1 and 2).

In the young plums, before corky material has been formed, the germ tubes also enter through the stomata. After entering they come in contact with normal fruit tissue, and direct infection takes place (Pl. XXXVIII, fig. 4). In all, 44 instances of infection through stomata or lenticels were noted, and although the surface of both ripe and green plums was often

well covered with germinating spores, no instances were found in which the germ tubes gained entrance directly through the cuticle.

Further evidence that the germ tubes do not usually penetrate the cuticle was obtained when two green plums of B×W 15, a very resistant variety, were scraped lightly with a sharp knife, thereby removing the cuticle without otherwise injuring the epidermis, and were then inoculated. These, with seven others of the same variety which had not been so treated, were sprayed with distilled water containing chlamydospores and put under a bell jar. At the end of 58 hours the two plums which had been scraped showed 10 and 13 spots, respectively, but rotted very slowly from the infection points. The seven unscraped plums were at this time without infection spots, but eventually three of these showed evidences of infection.

Because of this method of infection, resistance can not be attributed entirely to morphological differences in the epidermis of the varieties. There are however, certain morphological differences in the stomata and lenticels which contribute to resistance, the nature of which will be discussed later. When once the fungus has gained entrance the plums always rot more or less rapidly, depending upon the variety.

FIELD OBSERVATIONS

It is apparent from the facts given that the small amount of rot found in the orchard on green plums is not due to any greater resistance to infection which the green fruit may possess over ripe fruit. Nevertheless, the brown-rot in the orchard causes the greatest damage as a ripe-rot rather than as a green-rot.

It is a fact of considerable importance that it is not until the plums are ripe and begin to soften slightly that the fungus does its greatest damage as a ripe-rot. This is due probably to two reasons. The first is that there are greater possibilities of infection at this time. Field observations show that green plums will rot on the trees, owing usually to infection through curculio or other wounds, and that the rot will spread from one to another where they are in contact. Thus the number of rotted fruits and hence of infection sources to the ripe fruit is gradually increasing. Although there are other methods of infection, the largest number in ripe fruit is due directly or indirectly to contact with rotten green plums. It is very common in the field to find large groups of plums on a tree completely rotted, while other groups on the same tree are entirely free from rot. In these groups it is nearly always possible to trace the original source of infection back to one plum which has in most cases been infected through a wound of some kind while still green.

Another source of infection, more common in ripe or nearly ripe fruits than in green fruits, is direct infection from spore suspensions in water,

due probably to the greater number of spores being produced. This is not of considerable importance, however, except under extremely favorable weather conditions, when it may be the cause of a great deal of damage to fruits (Smith, 1889). A source of infection, common in completely ripened fruits and not common to green fruits, is through wounds caused by the cracking of the plums. This cracking is due either to excessive rainfall after a dry period, causing a rapid increase in turgor with the consequent splitting of the fruit, or to water remaining between plums which are in contact. This effect was also noted when ripe plums kept in a moist chamber cracked where they were in contact with the glass if water was present.

The second reason for the ripe-rot effect is the fact that the ripe fruit of some varieties is much more susceptible to rot after infection takes place than the green ones (see p. 388).

VARIETAL RESISTANCE OF PLUMS TO THE FUNGUS

That plums and peaches vary in their resistance to brown-rot has been noted from time to time. This power of resistance has been ascribed to various causes, such as a thick skin in certain varieties of resistant plums, a small amount of down on resistant peaches, and late ripening of some varieties, with consequent avoidance of the disease because of temperature conditions.

During the summer of 1913 attempts were made to determine whether definite differences in resistance to the brown-rot fungus really exist in plum varieties. Inoculation tests were started as early as June 14, when the plums were about one-third grown, and carried through on some varieties until maturity. Infection was brought about at first by spraying the plums with distilled water containing the spores. Later, a more effective method was found to be that of placing the plums in contact with moistened mummies well covered with spores. In both cases the experiments were carried on under bell jars in the laboratory.

RELATIVE RESISTANCE OF VARIETIES

Table IV shows the relative resistance of varieties as determined by the inoculation of 262 plums through uninjured skin and the subsequent rotting of the fruits.

The skin and flesh descriptions, except where indicated, were taken from a table prepared by Dr. M. J. Dorsey, of the Minnesota Experiment Station, in a study of "fruit characters" in hybrid plums, prepared independently of the investigations on resistance. The descriptions of varieties indicated by an asterisk (*) were made by the writer.

TABLE IV.—Texture of flesh and skin, ripening date, and relative resistance of varieties of plums to *Sclerotinia cinerea*

Variety.	Date of ripening.	Texture of flesh.	Texture of skin.	Thickness of skin.	Relative susceptibility. ^a
A × W 2	Aug. 25	Medium firm, tender. . .	Medium.	Medium. .	
A × W 11	Aug. 19	Firm, medium tender. .	Tough. . .	Thin.	++
A × W 12					+++
A × W 15	Sept. 2	Firm, tender.	Tough. . .	Medium. .	++
A × W 17	Aug. 18	Tender.	Medium. . .	do.	
A × W 18	Sept. 2	Medium firm, tender. . .	Tough. . .	Medium. .	+
B × W 1	Aug. 31	Soft, tender.	Medium. .	Medium. .	++
B × W 2	Aug. 31	do.	Tender. . .	Thin.	+++
B × W 4	Sept. 2	do.	Medium. .	Medium. .	++
B × W 5	Sept. 2	Medium firm, tender. . .	do.	Medium. .	++
B × W 6	Aug. 22	Firm, tender.	Tough. . .	Medium. .	++
B × W 9	Aug. 31	do.	do.	do.	+
B × W 12	Aug. 18	do.	do.	do.	++
B × W 15	Sept. 2	Very firm, medium tender.	Medium. .	Thick. . .	+
B × W 16	Aug. 27	Soft, tender.	do.	do.	++
B × W 21	Aug. 19	Firm, tender.	Tough. . .	do.	++
*S. D. No. 1.	Aug. 15	Soft, tender.	Tender. . .	Thin.	++++
*S. D. No. 2.	Aug. 15	Firm, tender.	Medium. .	Medium. .	+
*S. D. No. 3.	Aug. 15	do.	do.	do.	+
Burbank.	Aug. 17	Soft, tender.	Tough. . .	do.	+++
Wolf.	Sept. 1	Medium firm, tender. . .	Medium. .	Thick. . .	++
*Ocheeda.			Tough. . .	Medium. .	++
*Harrison.			do.	do.	++
*Surprise.			Medium. .	Thick. . .	+++
*Hammer.			do.	Medium. .	+++
*Newman.			do.	do.	+++
*Manitoba No. 1.			Tender. . .	do.	++++
*Americana seedling No. 1.		Firm, tough.	Tough. . .	Thick. . .	+
*Americana seedling No. 2.			do.	Thin.	++++
Etopa.		Soft, tender.	Tender. . .	do.	++++
Opata.	Aug. 17	do.	Medium. .	do.	+++
Okiya.	Aug. 18	do.	Tender. . .	do.	++++
Wakapa.	Aug. 18	do.	do.	do.	++++
Compass.	Aug. 15	do.	do.	do.	++++
Sand cherry.	Aug. 10	do.	do.	do.	++++
*Reagan.	Sept.	Very firm, medium tender.	Tough. . .	Thick. . .	+

^a + Indicates least relative susceptibility; ++++ indicates greatest relative susceptibility.

The results show striking differences in resistance of the several varieties to infection. In the case of very susceptible varieties, as the Compass and sand cherry, it is always very easy to get a large number of infection spots. In the case of a very resistant variety, such as B × W15, it is often very hard to cause infection. In one trial, begun on July 8, 1913, in which green plums, about three-quarters grown,

were inoculated by contact with mummies in a moist chamber, the following results were noted after 27 hours:

Variety.	Number of plums.	Points of contact.	Number of infection spots.
B × W16	1	1	Many.
B × W2	1	1	Do.
Burbank	1	1	20.
B × W15	4	6	None.
Topa	1	1	1.
Opata	1	1	Many.

Another trial with B × W15, directly following this and carried on under the same conditions, showed a few infection spots in three out of five contact points, indicating that in some cases the fungus can enter these resistant plums. A number of other experiments, comparing the relative resistance to infection of B × W15 with that of other varieties, showed results comparable to those given above.

Soon after infection takes place a small decayed spot appears on the surface of the plum. These spots increase in size rapidly in the susceptible varieties and soon completely cover the plum. This often requires not longer than 24 hours after infection has taken place. On the resistant forms, however, the spots increase in size slowly, sometimes taking several days before they entirely cover the plum. The rapidly rotting plums take on the characteristic brown color of rotten fruit; but the slower rotting varieties often become dark blue and when completely rotted become black.

Usually when the susceptible varieties are one-half to three-quarters rotted, they begin producing tufts of chlamydospores over the rotted area. On the sand cherry and some of the sand-cherry hybrids, which are very susceptible, the spore tufts are usually large and numerous (Pl. XXXVIII, fig. 9). Varieties such as B × W21, which appear intermediate in the rapidity with which they rot, usually produce spore tufts, but they are nearly always smaller and less numerous than those on the susceptible varieties (Pl. XXXVIII, fig. 7 and 8). In the case of the most resistant varieties it is seldom that spores are produced if the skin has not been broken. If the plum has been wounded, spores are usually produced through the wound (Pl. XXXVIII, fig. 6). Under particularly favorable conditions pustules may appear through the uninjured skin, in which case they are usually small, and few in number.

RELATION OF SKIN THICKNESS TO RESISTANCE

In order to determine the part played by thickness of skin in resistance, inoculations were made by cutting off a small piece of skin and planting

the spores on this freshly cut surface of the plum in a drop of water. The plums were kept in a moist chamber. The same relative differences in rapidity of rotting were noted in these cases as when the infection took place through the uninjured skin, indicating that mere thickness of skin is not the deciding factor in resistance, as the cells underlying the skin show the same relative resisting powers.

However, it will be seen by referring to Table IV that the varieties which are the most susceptible are the thin-skinned, tender-fleshed ones, while the more resistant varieties are thick-skinned and of a firmer, tougher texture. An examination of prepared slides of the skin of the different varieties confirms these observations, in that all of the very susceptible varieties have a thin skin (Pl. XXXVII, fig. 4), consisting of one or two layers of cells besides the epidermis; while the resistant varieties all have a very thick skin (Pl. XXXVIII, fig. 4), consisting of from five to eight layers of cells. The varieties appearing to be intermediate in resistance have skins varying in thickness, but in all cases examined they are thicker than the susceptible forms. It would seem, then, that there is a rather close correlation between skin thickness and resistance to the brown-rot fungus.

RELATION OF STOMATA AND LENTICELS TO RESISTANCE

In studying the method of infection, a comparison of the stomata and lenticels of the different varieties revealed some interesting and important facts relating to resistance. The lenticels described above, in which no change other than the production of a few flat cells lining the cavity (Pl. XXXVII, fig. 1) took place, were found only in the thin-skinned varieties, as Gold and some of the sand-cherry hybrids. Those in which the lining cells became corky (Pl. XXXVII, fig. 3) were found in the thicker skinned varieties.

In two of the most resistant varieties, B×W15 and A×W9, the formation of lenticels, due to filling of the stomatal cavity with parenchyma cells, was very common (Pl. XXXVII, fig. 4). This condition was not entirely confined to these varieties, as instances were found in many others of the thick-skinned varieties and also in such a thin-skinned variety as Gold (Pl. XXXVII, fig. 2 and 4), where, however, only a few cells were formed that did not in any case completely fill the cavity (Pl. XXXVII, fig. 2).

That the complete plugging of the stomata is a factor in resistance is shown by the fact that many instances were noticed in which these stomata were completely covered by germinating spores, with no resulting infection. It did take place, however, through stomata the cavities of which were only partially filled with these cells and also through those in which only the corky tissue was present (Pl. XXXVIII, fig. 1, 2, and 5). This may explain why it was possible to obtain so few infections in A×W9 and B×W15, even when their surfaces were covered with germinating spores.

PHYSIOLOGICAL RELATION OF FUNGUS TO HOST

That resistance is not entirely due to the partial inability of the fungus to gain entrance to the tissues of the resistance forms is shown by the difference in rapidity of rotting after infection has taken place. A study of the further penetration of the fungus in the resistant and susceptible forms was therefore undertaken.

Previous investigators do not agree as to the manner in which the fungus penetrates the host tissues, some holding that it penetrates the cell walls wherever it comes in contact with them and that it shows no particular affinity for the middle lamella (Cooley, 1914), while others hold that the fungus follows the middle lamella and may or may not split it completely (Schellenberg, 1908; Bruschi, 1912).

The method used in the present study of the relation between the host and the fungus cells was the same as that used in the determination of the method of infection—i. e., a study of prepared slides of infected plum and apple tissue. The stains already mentioned were used. The material consisted of small blocks of plum and apple tissue cut from the edge of the rotting spots and also blocks cut from plums which had been infected within 12 to 30 hours of the time of killing. For this study of the penetration of the fungus, over 220 slides were prepared from material collected from 17 varieties of plums and 4 varieties of apples. In 80 of these slides the fungus hyphæ were clearly differentiated from the host tissue.

PENETRATION

In all cases the fungus shows a very strong affinity for the middle lamella (Pl. XXXVIII, fig. 2, and XXXIX, fig. 1, 2, 5, and 6). No instances were found where the hyphæ had actually pierced the cell walls and entered the cell cavity, so that it seems certain that the hyphæ of *S. cinerea* are unable to penetrate the cell walls of the plum and apple fruits. No record has come to notice of other investigators having extracted from the brown-rot fungus a cellulose-splitting enzyme which has the power of dissolving the plum cell walls. Furthermore, that such an enzyme is not produced by the fungus in the host tissues is clearly demonstrated by the fact that in completely rotted plum tissue (Pl. XXXIX, fig. 5) and in sclerotia which have been buried in the ground for over 18 months and have produced apothecia, the cell walls are still intact.

From the appearance of the infected tissue it is evident that the fungus hyphæ secrete a substance which splits out the middle lamella slightly in advance of its penetration through the tissue (Pl. XXXIX, fig. 1, 2, 3, 5, and 6). Eventually the middle lamella is completely dissolved, leaving the cells in the rotted area entirely free from one another. Instances comparable to those illustrated were found in nearly all of the slides examined.

The killing of the host cells, so far as is revealed by the microscopical examination, seems due principally to a modification of the osmotic relations of the cells as a result of the disappearance of the middle lamella and to much of the liquid contents of the cells being withdrawn by the fungus to be used in its development. In the plum the chloroplasts and chromoplasts contained in the cells lying directly under the epidermis appeared not to be disintegrating in those cells which had not so collapsed as to make observation impossible. The cytoplasm of the deeper-lying cells was very scant, but showed evidences of plasmolysis, often unmistakably in advance of the penetration of the hyphæ (Pl. XXXIX, fig. 3).

MIDDLE-LAMELLA SOLVENT

The nature of the substance secreted is not at all clear. From the effect on the host tissue it would appear that the middle-lamella-dissolving enzym pectinase was produced, but attempts to isolate it were without success.

Juice was pressed from rotten portions of apples and loquats (*Eriobotrya japonica*) infected with the brown-rot fungus. This was filtered under sterile conditions, in some cases through coarse, and in others fine filter paper. Slices of healthy apple and loquat fruits were partially immersed in the liquid, but showed no softening effect in any case after several days. Further trials with a method to be described later, used in separating pectinase from *Penicillium expansum*, also gave negative results with *S. cinerea*.

In another case a partially rotted apple plug was put into a test tube on cotton above commercial formalin so that the plug did not come in contact with the liquid. It was thought that the fungus would be killed by the fumes, but that if a pectinase were present it would continue to rot the tissue. No further rotting took place, and at the end of five days the tissue, unaffected at the beginning, was still firm and of normal color.

An attempt was made to isolate the enzym pectinase from a culture of *S. cinerea*, 86 days old, on apple cider. The method used was that described by Pringsheim (1910), which consists, in brief, of thorough drying of the material with acetone, followed by pulverization of the dried material and extraction of the enzym with a small quantity of water. On May 8, 1915, succulent twigs of B×W21 plum, sand cherry, and pear (*Pyrus betulifolia*) were partially immersed in the liquid extract in test tubes; also pieces of ripe apple the flesh of which was slightly mealy, and pieces of young peaches, one-quarter grown, were entirely immersed. The tubes were placed in a constant-temperature oven at 35° C. Checks were run, using water in place of the extract.

After 24 and 48 hours the plum, pear, and sand-cherry twigs showed no effects from the treatment other than a slight wilting. The tissues were not softened. The blocks of green-peach fruit showed no softening. After 15 hours the apple plug had softened slightly over the surface, but

was still firm in the center. After 48 hours it had softened completely. A portion not immersed in the liquid, but which came in contact with it at one point, was softening from this point and becoming discolored. The checks in water remained firm and were not discolored.

Although the effect of the extract on the apple tissue appeared to be that of a pectinase, it can hardly be concluded that this enzyme was present, as the fruit used was overripe and slightly mealy, and could very easily have been broken down by other solvents contained in the extract.

DeBary (1886) considered the possibility of oxalic acid being the toxic substance produced by *S. libertiana*, because he found the hyphæ often coated with crystals of it; however, he later discarded this notion for the reason that solutions of oxalic acid did not give the same effect as the fungus. Smith (1902) extracted a substance from *Botrytis cinerea*, which, whether boiled or unboiled, caused a rot of the host tissue identical with that caused by the fungus. He concluded it was not an enzyme, but that the effect might be due to oxalic acid, which he found to be present in quantities often as high as 2 per cent. Peltier (1912) confirmed the results regarding this action of the extract, but was unable to detect the presence of oxalic acid, even in old cultures.

The possibility of oxalic acid being the toxic substance of *S. cinerea* was considered, as Cooley has demonstrated that it is produced in appreciable amounts in cultures of *S. cinerea* on plum and peach juice, and in peaches which had been rotted by the fungus. In order to determine the effect of oxalic acid on vegetable tissue, small blocks of onion, potato, tomato, dahlia, radish, coleus (young shoot), tomatoes (young shoot), loquat (fruit), canna (bulb), oxalis (petiole), geranium (young shoot), and apple were immersed in 0.015, 0.062, 0.125 per cent solutions of oxalic acid and the effect noted at the end of 24 and 48 hours. In all of the solutions the apple, loquat, and oxalis softened, while in the 0.125 per cent solution only the onion and tomato softened slightly. The potato did not soften even in 0.25 per cent solution. In all cases bleaching occurred. An examination of the different tissues showed that the softening was due to the solution of the middle lamella.

The fact that oxalic acid even in such dilute solutions readily softened the tissues of the apple and loquat, upon both of which the brown-rot grows readily, might indicate that the oxalic acid was the toxic substance, but the bleaching effect produced by the acid and the fact that when used even as strong as 0.25 per cent it had no effect on potato, upon which the fungus also grows readily, would seem to indicate that this acid is not the sole toxic substance produced.

COMPARISON OF FIRM-ROT AND SOFT-ROT

Cooley (1914) pointed out the very interesting fact that, although *P. expansum* and *S. cinerea* apparently acted differently on their hosts, the one producing a soft-rot of fruits and the other a firm-rot, in culture

they gave identical results when grown on media containing cellulose, from various sources, or calcium pectinate. They were able in certain cases to hydrolyze the cellulose, but showed no dissolving action on calcium pectinate.

In order to determine the difference between a soft-rot and a firm-rot caused by fungi which physiologically were acting alike in culture, apples rotting from *P. expansum* were examined. A smear of the rotted tissue revealed the fact that the host cells were entirely separated from one another, but that the walls were apparently intact. A few very small hyphæ could be seen, seeming to be entirely intercellular. Further examination of prepared slides of material, taken both from the oldest portion of a spot 3 inches in diameter and from the edge of the rotting spot, confirmed the above observations. The middle lamella was completely split out between all of the cells in the rotted area, and the cellulose walls were entirely intact. The few very small hyphæ that were found were intercellular (Pl. XXXIX, fig. 4). So far as could be seen, the two fungi, *S. cinerea* and *P. expansum*, act in exactly the same way on the host tissue. The reason for one causing a firm-rot and the other a soft-rot is not, then, due to any differences in physiological action, but appears to be merely mechanical, due to the fact that *S. cinerea* completely fills the intercellular space produced by the collapse of the cells (Pl. XXXIX, fig. 5), with very large hyphæ, while *P. expansum* produces few small hyphæ, which give little support to the host tissues, and, as a consequence, they collapse as the rot proceeds (Pl. XXXIX, fig. 4).

The complete solution of the middle lamella in tissue rotted by *P. expansum* would seem to indicate the presence of a middle-lamella-dissolving enzym. To test this, squares of very fine-grained filter paper were laid on blocks of apple and small portions of flesh from the edge of the rotting spot were laid on the filter papers. All precautions were observed, in order to keep the materials sterile. It was thought that if a pectinase were present it would filter through the paper and cause a soft-rot of the fruit. The papers bearing the rotted flesh were removed after $3\frac{1}{2}$ hours. In four cases out of seven, infection took place through the filter paper and the normal soft-rot followed, while in the three other cases the blocks became soft and translucent at the end of two days, but showed no signs of infection. A microscopic examination showed the cells to be separated from one another, owing to the complete solution of the middle lamella. The checks remained firm. A small portion of the tissue, which rotted in the absence of hyphæ, when transferred to the checks caused them to rot rapidly. This and the fact that in the typical rot spots the middle lamella is completely dissolved in the presence of very few hyphæ would indicate that *P. expansum* secretes a very active middle-lamella-dissolving enzym, pectinase.

RESISTANT AND SUSCEPTIBLE VARIETIES

The fungus hyphæ of *S. cinerea* in both resistant and susceptible fruits show practically no constant differences. In both cases they are large and densely protoplasmic over their entire length. In a few instances hyphæ in resistant forms appeared more knotted and irregular than in susceptible ones, but this could be explained in those cases by mechanical pressure of the small cells of the hypodermal layer, which in the resistant plums appear to be less easily collapsed than in the susceptible varieties. Considerable difference, however, could be noticed in the rapidity with which the hyphæ developed in the two forms. The hyphæ in the susceptible varieties usually completely filled the intercellular spaces as the rot spread, while in the resistant ones fewer hyphæ were produced. A few instances were noticed in resistant varieties of cells lying completely or nearly completely surrounded by hyphæ from which the middle lamella had not been dissolved. This and the fact that in these forms the middle lamella seldom appeared to be dissolved out far ahead of the penetration of the fungus lead to the conclusion that this partial resistance is due to the inability of the toxic material secreted to dissolve the middle lamella as rapidly in the resistant as in the more susceptible varieties, owing possibly to very slight differences in its composition.

That there is an actual difference in the composition of the middle lamella material seems fairly certain. It is well recognized that varieties of plums, apples, and other fruits and vegetables vary greatly in the time required for cooking. Some remain firm after a long period of boiling, while others soften and become mushy after very short heating. An examination of boiled-apple tissue which had become soft revealed the interesting fact that the softening was due in part to a separation of the cells as a result of the middle lamella having been dissolved. The cell walls appeared not to be ruptured at all. In those varieties which do not become soft on boiling it is assumed that the middle lamella material is less soluble and therefore is probably of a slightly different chemical composition. It is recognized, of course, that the dissolving action of the fungus upon the pectic substances and solution by hot water are entirely different processes and, therefore, resistance to the fungus and firmness after cooking may or may not be correlated.

In view of the fact that eventually in both resistant and susceptible forms the middle lamella is completely dissolved, the difference in sporulation (Pl. XXXVIII, fig. 6, 7, 8, and 9), as described above, could hardly be explained by variations in middle lamella composition, but rather points to a small amount of some toxic substance being produced either by the host cells or fungus hyphæ, which is not enough to completely stop the growth of the fungus, but merely to retard slightly its normal functioning.

TOXICITY OF ORGANIC ACIDS TO THE FUNGUS

In a series of tests carried on by the writer to determine the relative toxicities of the fruit acids to *S. cinerea*, results were obtained with regard to oxalic acid which may throw some light on the cause of these differences in sporulation. Hanging-drop cultures containing large numbers of the chlamydospores in suspension in solutions of oxalic, tannic, gallic, tartaric (inactive), malic, and citric acids were used. In all of the tests the oxalic-acid solutions were found to be by far the most toxic. As has been noted, Cooley (1914) found this acid to be produced in appreciable quantities by the fungus in culture. In view of this, it is very possible that in the slow development of the fungus in the resistant fruits enough oxalic acid is produced by the hyphæ to actually become toxic to them, resulting in the production of few or no spore tufts.

RIPE-ROT

The discussion of the penetration of the fungus thus far has had special reference to green and ripening plums, but not to those plums which have begun to soften slightly as a result of the ripening process. It is when the plums begin to soften that the fungus works the greatest havoc, and it is then that variations in resistance are most noticeable in the orchard.

Cook and Taubenhaus (1912) were able to demonstrate a positive correlation between the decrease in the oxidizing-enzyme content of the fruits of many plants, due both to maturing and to removal of the fruit from the plant, and a decrease in their resistance to certain diseases. They could show no correlation between acid content of apples and pears and resistance to disease. Cooley (1914) was able to confirm these latter results in the plum, finding that as the plums matured the acid content increased until it reached its maximum at the time of ripening of the fruit, which was also the period of greatest susceptibility to the brown-rot fungus. As acidity will not explain the decrease in resistance of plums to the rot on ripening, can it be explained by a decrease in the oxidizing-enzyme content of the plums?

Ripe fruits of the Reagan plum, which is a resistant variety, were sent to this Station from New York on October 22, 1914. On November 7 they were inoculated with the brown-rot, both by spraying on spores and by laying the plums in contact with moistened mummies. By this time the oxidizing enzyme should have entirely disappeared, owing both to ripening and to removal from the tree. In spite of this, the plums were found to be still very resistant both to infection and to rot after infection occurred. It is evident then that resistance can not be due in this case to the presence of the oxidizing enzyme.

Material of these plums was sectioned, and it was found that in the healthy tissue of these very ripe plums the middle lamella was still

present (Pl. XXXVII, fig. 6). The plums at the time of preserving the material (Nov. 7, 1914) were firm. An examination of the healthy tissue of ripe susceptible varieties revealed the fact that the middle lamella in these was completely dissolved (Pl. XXXVII, fig. 5). These plums were soft when the material was fixed. That the pectic-acid compounds change to pectin in the ripening fruit is a well-known fact. In view of the fact that the brown-rot can only spread after the middle lamella has been dissolved, the reason for the increase in susceptibility on ripening in those varieties which become soft as a result of the normal loss of the middle lamella owing to ripening is readily seen.

The reduced possibilities of infection owing to the plugging of many of the stomata, the causes of which have already been explained, and the persistence of the middle lamella after ripening, as shown by the fact that the fruits remain firm, explain the resistance to brown-rot of such varieties as Reagan, B×W15, B×W9, S. D. Nos. 2 and 3, and Americana Seedling No. 1.

RELATION OF TANNIN CONTENT OF THE HOST TO RESISTANCE

A great deal of attention is being given to the relation between chemical substances within the host cell and resistance. The work of Comes (1913) on the correlation between the increased acid content in wheat plants and rust resistance has been mentioned. Cook and Taubenhaus (1911) were able to show that tannin, a very common product in plants, was toxic in varying degrees to many fungi in culture and considered that it might be a very important factor in resistance. Bassett and Thompson (1911) showed that apples and pears contain an oxidizing enzyme capable of producing from gallic acid a tannin-like substance having the power of precipitating protein from solution. They found this product to be toxic to "a fungus." The juices of green apples, pears, and walnut hulls (unboiled) produced a substance which on standing precipitated soluble protein from the juice. They considered this to be a tannin-like substance and to be controlled by the oxidizing enzyme.

If the tannins disappear on the ripening of the fruit, as is generally supposed, we may have an explanation of the greater susceptibility of some fruits to disease on ripening. The evidence of the disappearance of tannin on ripening, however, is not at all conclusive. One of the most striking instances of its apparent disappearance is that of the persimmon (*Diospyros virginiana*), the green fruits of which are very astringent, while the ripe, soft fruits are not at all astringent. Gore (1911), however, showed that the tannin did not disappear, but was inclosed in sacs which broke readily in green fruits in contact with saliva, but were not affected in the ripe fruit. Similar structures have been observed in the carob-bean pod (*Ceratonia siliqua*) and in the date fruit. Bassett and Thompson (1911) demonstrated that "apples that had fallen from

the tree showed about twice as much tannin as those freshly plucked." It is a matter of common observation that some plums, especially the sand cherry, contain considerable amounts of an astringent substance, probably tannin, even when dead ripe. It is not altogether clear, therefore, that the disappearance of the tannin on ripening is a cause of the increased susceptibility of ripe fruits to rot.

There is still the possibility that differences in resistance of varieties may be due to unequal tannin content. In order to determine this point, tannin determinations were made of the fruit of 11 varieties of plums. The method used was Proctor's modification of Lowenthal's method as described by Leach (1913, p. 370). The results given in Table V are for tannin substances calculated as gallotannic acid. The determinations were made on fruit which had been picked 14 hours, except in the case of the sand cherry and Compass, which were made directly after picking.

TABLE V.—*Tannin content of ripe and green plums on August 6, 1915*

Variety.	Condition.	Date of ripening.	Percentage of tannin in pulp.	Percentage of tannin in dry matter.	Percentage of dry matter.	Relative susceptibility.
Sand cherry.....	Ripe.....	Aug. 1	2.087	15.081	13.84	+++ +
131 X (sand-cherry hybrid).	...do.....234	1.483	15.75	+++ +
Compass X pin cherry.	...do.....338	2.388	14.17	+++ +
Sapa.....	Turning....	Aug. 17	.362	3.367	10.75	+++ +
Compass.....	Green.....	Aug. 15	.483	4.229	11.42	+++ +
A X W12.....	...do.....482	3.418	14.10	+++ +
Opata.....	Turning....	Aug. 17	.733	4.618	15.87	+++ +
Burbank.....	Green.....	...do....	.185	1.516	12.20	+++ +
B X W21.....	...do.....	Aug. 19	.773	5.777	13.38	+++ +
A X W15.....	...do.....	Sept. 2	1.131	9.520	11.88	+++ +
Americana Seedling No. 1.	...do.....665	3.873	17.17	+++ +

The relative-susceptibility determinations were made at the same time as the tannin determinations and are confirmed by previous tests on some of the varieties and by field observations on all of them.

It is readily seen that very little relationship exists between tannin content and resistance to the brown-rot fungus. Even though a correlation could be shown between tannin content and resistance, it still remains to be proved that the tannin is an actual factor in resistance, since the following facts indicate that it does not come into direct contact with the fungus hyphæ. The hyphæ are apparently always intercellular, and according to Haas and Hill (1913, p. 192)—

In the cell the tannin occurs in solution in the cell sap, and since tannin forms a precipitate with albuminous matter it follows that the layer of protoplasm around the tannin vesicles must be impermeable to it; if this were not so the protoplasm would be tanned on the production of tannin.

CONCLUSIONS

(1) The brown-rot fungus in Minnesota seems to be identical with that found in other parts of this country and with *Sclerotinia cinerea* of Europe. Chlamydospore tufts vary in color from gray to bright ocher. For the production of the ascus stage the sclerotium apparently must be buried in the ground for two winters. Mummies which have hung on the trees for one year are still capable of producing apothecia.

(2) Infection may take place through the uninjured skin at any time during the development of the plum fruit. The hyphæ enter through the stomata and lenticels. Varieties show great differences in resistance to infection, owing to the production of parenchymatous plugs which fill the stomatal cavity and to lenticels made up of layers of corky cells through which the hyphæ are unable to penetrate. Corky cells lining the stomatal cavity merely delay infection.

(3) Varieties show variations in resistance to rot after the hyphæ have gained entrance. Resistance is apparently correlated with (a) a thick skin; (b) the production of parenchymatous plugs which fill the stomatal cavity; (c) the production of corky walls in the lining cells of the stomatal cavity; and (d) firmness of fruit after ripening. There seems to be no relationship between oxidase content of the fruit and resistance or between tannin content and resistance.

(4) Brown-rot is essentially a ripe-rot, affecting the plums most noticeably as soon as they begin to soften slightly as a result of ripening. Varieties which are resistant remain firm on ripening. Softening during ripening is due to the solution of the middle lamella.

(5) The hyphæ of *S. cinerea* in the tissue of plum and apple fruit are entirely intercellular. The middle lamella is dissolved slightly in advance of the penetration of the hyphæ. The absence of the middle lamella in fruits which have softened owing to ripening explains the greatly increased spread of the disease at ripening time. Attempts to demonstrate the presence of the middle-lamella-dissolving enzym, pectinase, in rotting fruits or to extract it from a culture of the brown-rot fungus on apple cider proved futile.

(6) The rot caused by *S. cinerea* is a firm-rot due to the mechanical support of the hyphæ which completely fill the intercellular spaces left by the collapse of the host cell walls. *Penicillium expansum* produces a soft-rot, because of the fact that few hyphæ are produced and, therefore, little mechanical support is given to the rotted tissue, which as a consequence collapses as the rot proceeds. The hyphæ of *P. expansum* are intercellular and produce a substance which dissolves the middle lamella even in the absence of the fungus hyphæ.

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PLATE XXXVII

Fig. 1.—Lenticel in ripe fruit of Sapa plum. The walls of the cells lining the cavity give the staining reaction of cellulose. $\times 400$.

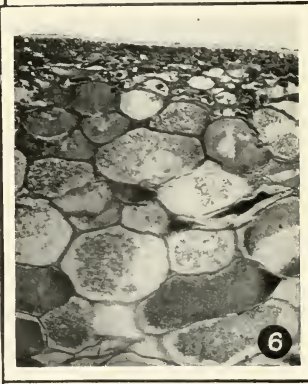
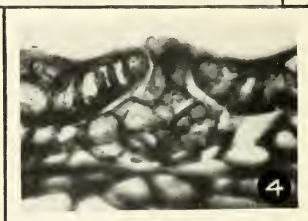
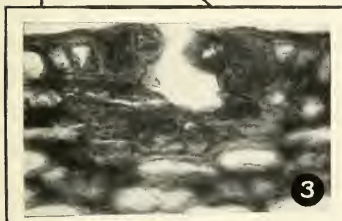
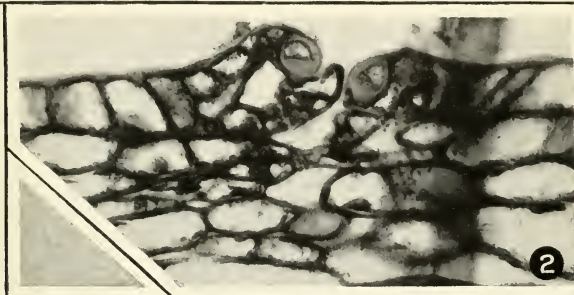
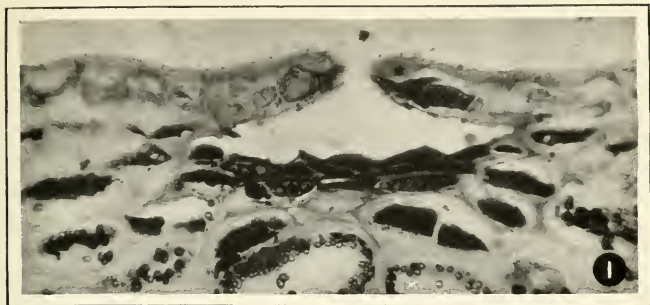
Fig. 2.—Lenticel in ripe fruit of Gold plum partially filled with parenchymatous cells. Infection may take place through a lenticel of this type. $\times 400$.

Fig. 3.—Lenticel in green Burkank plum. The cell walls lining the cavity give the staining reaction of cork. Infection may take place through a lenticel of this type, but only in the manner shown in Plate XXXVIII, figures 1, 3, and 5. $\times 400$.

Fig. 4.—Lenticel in green fruit of B \times W 21 completely filled with parenchymatous tissue. Infection can not take place through a lenticel of this type. $\times 400$.

Fig. 5.—Ripe healthy tissue of Sapa plum, showing middle lamella completely dissolved out owing to ripening process. This is the condition found in the ripe fruits of the susceptible varieties. $\times 60$.

Fig. 6.—Ripe healthy tissue of Reagan plum two weeks after picking. The middle lamella is still intact. This is the condition found in the ripe fruit of resistant varieties. $\times 60$.



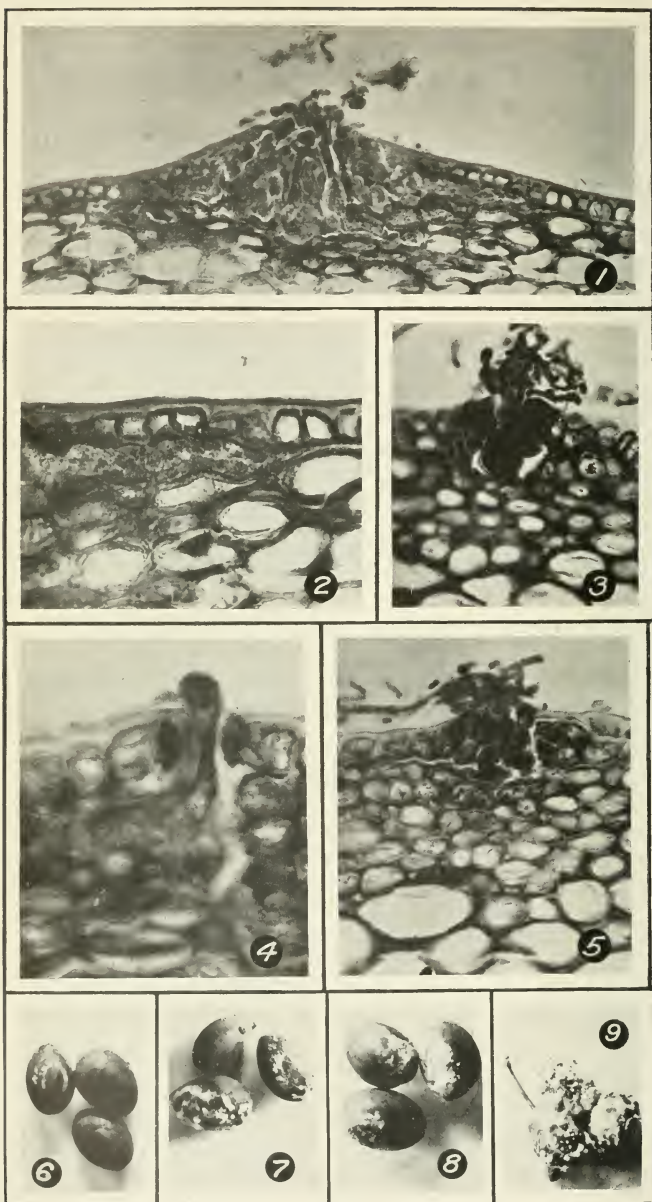


PLATE XXXVIII

Fig. 1.—Infection through a lenticel of Burbank plum the cavity of which is lined with corky-walled cells. The hyphæ are incapable of dissolving the middle lamella between these cells, but apparently exert enough pressure to split the epidermis away from the underlying cells, thereby allowing the hyphæ to enter the fruit tissue. $\times 216$.

Fig. 2.—Left side of figure 1 in detail, showing hyphæ entering the fruit tissue after the epidermis has been raised by the growth of the hyphæ in the stomatal cavity. $\times 400$.

Fig. 3.—Infection through a lenticel in B \times W4. The hyphæ swell on entering, filling up the stomatal cavity. $\times 200$.

Fig. 4.—Infection through a stoma in a young green fruit of *Prunus americana* seedling No. 1, in which no corky walls have yet been formed. $\times 400$.

Fig. 5.—Infection through a lenticel of the same type as is shown in figures 1 and 3. The hyphæ have filled the stomatal cavity and are raising the epidermis from the underlying cells. The hyphæ can enter the fruit tissue through the split thus formed. $\times 200$.

Fig. 6.—Half-grown fruits of B \times W15 completely rotted through wound inoculations. Only very few spore tufts are being produced. This is a resistant variety.

Fig. 7.—Half-grown fruits of B \times W21 completely rotted through wound inoculations. This variety is intermediate in degree of resistance.

Fig. 8.—Half-grown fruits of A \times W15 completely rotted through wound inoculations. This variety is intermediate in degree of resistance.

Fig. 9.—Half-grown fruits of Etopa plum completely rotted through wound inoculations. The plums are completely covered with large spore tufts. This is a very susceptible variety.

PLATE XXXIX

Fig. 1.—A rotting area in an overripe fruit of S. D. No. 3. In the healthy portion at the right the middle lamella is still intact, while in the rotted portion the cells are free. This is a resistant variety. $\times 216$.

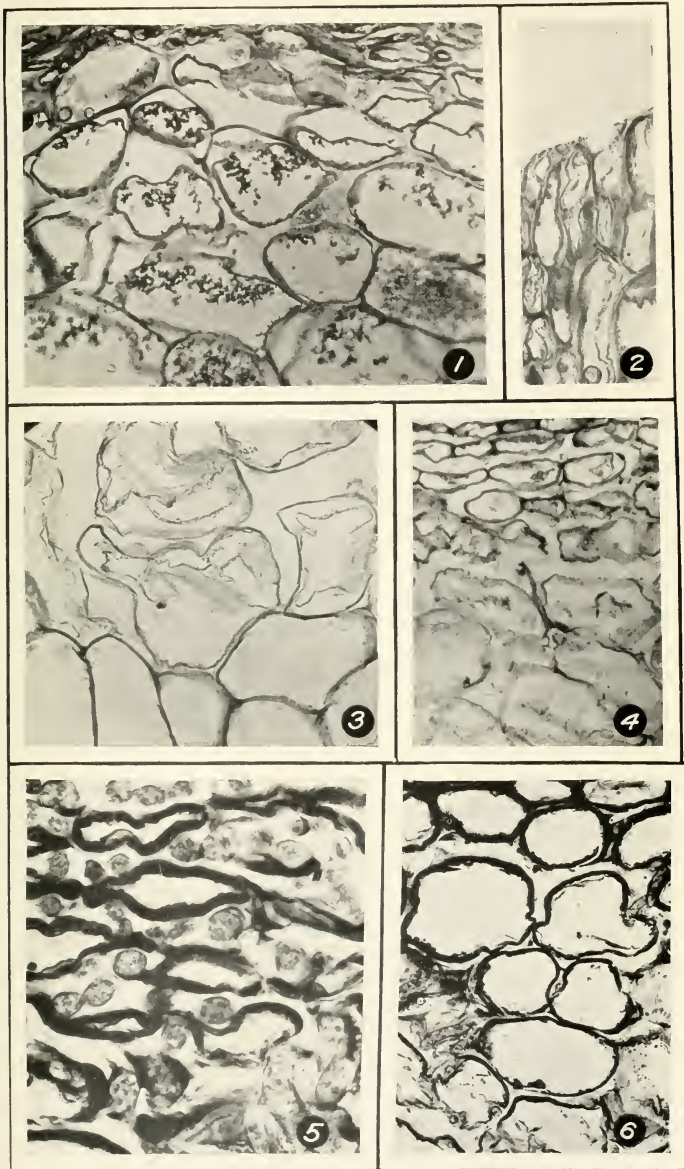
Fig. 2.—Tip of hypha in Oyata plum. The middle lamella is being split slightly ahead of the hyphæ. This is apparently not due to mechanical pressure, as the walls in contact with it are collapsed. $\times 200$.

Fig. 3.—The edge of a rotting spot in a green fruit of Oyata plum. The middle lamella is dissolved in advance of the penetration of the hypha. This is a susceptible variety. $\times 216$.

Fig. 4.—Tissue of apple infected with *Penicillium expansum*. A short piece of hyphæ may be seen in the center of the figure. The middle lamella is completely dissolved. $\times 156$.

Fig. 5.—Cross sections of hyphæ in tissue of Oyata plum 18 hours after inoculation. The dark areas are collapsing cell walls. The hyphæ are entirely intercellular. $\times 400$.

Fig. 6.—Portion of the rotted area of an Oyata plum 18 hours after inoculation. Although only few hyphæ are present, the middle lamella is completely dissolved. $\times 200$.



FREQUENCY OF OCCURRENCE OF TUMORS IN THE DOMESTIC FOWL,¹

By MAYNIE R. CURTIS,

Assistant Biologist, Maine Agricultural Experiment Station

The work of Rous, Murphy, Tytler, and Lange on the neoplasms of the domestic fowl has aroused some interest in the frequency of their occurrence. In the course of 10 months Rous, Murphy, and Tytler² obtained without difficulty about 30 spontaneous tumors in living fowls. On examining 4,000 hens brought to a hotel, Ehrenreich³ found 7 malignant tumors. All of these occurred in hens more than 1 year old, of which there were 1,000.

For the last 8 years it has been the routine practice at the Maine Agricultural Experiment Station to make autopsies on all birds that either die from natural causes or are killed by accident or for data. In making these autopsies it has been the uniform practice to record the presence of tumors, the organs in which they occur, and whether or not the tumor is of cystic or solid tissue structure. No further study has been made of any tumor. The data were collected primarily because of the possible effect of the presence of the tumor on the other data taken. In going over the records lately, however, their bearing on the frequency of the occurrence of neoplasms in fowls has seemed worthy of analysis and publication. The archives of the laboratory now contain 880 autopsy records sufficiently complete for use in this study.

Of the 880 birds on which autopsies were performed carefully, 79, or 8.98 per cent, had tumors of one sort or another. If we may consider these 880 birds a random sample of fowls as a whole, we may conclude that there are about 90 cases of tumors per 1,000 fowls. While these fowls are not a fair random sample, they are probably nearer one than any other equally large group on which data are at present available. It is possible, however, by the analysis of these records to study the frequency of occurrence of tumors in birds that die from natural causes compared to the frequency in normal birds that are killed. It is also possible to study the relation of the occurrence of tumors to age and sex.

It is a well-known fact that in man there are many tumors which do not primarily affect the health of the host. This seems to be equally true of fowls. Table I shows the occurrence of tumors, first, in birds that

¹ Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 86.

² Rous, Peyton, Murphy, J. B., and Tytler, W. H. A filterable agent the cause of a second chicken-tumor, an osteochondrosarcoma. *In Jour. Amer. Med. Assoc.*, v. 59, no. 20, p. 1793-1794. 1912.

³ Ehrenreich, M., and Michaelis, L. Ueber Tumoren bei Hühnern. *In Ztschr. Krebsforsch.*, Bd. 4, Heft 3, p. 586-591. 1906.

Ehrenreich, M. Weitere Mitteilungen über das Vorkommen maligner Tumoren bei Hühnern. *In Med. Klin.*, Jahrg. 3, No. 21, p. 614-615. 1907.

either died from or were killed because of disease, and, second, in apparently normal birds accidentally killed or killed for data.

TABLE I.—*Percentage of tumors found in birds dead from natural causes and in normal birds which were killed*

Manner of death.	Total number of birds.	Percentage of birds with tumors present.
Natural causes.....	660	8.94
Killed.....	220	9.09
Total.....	880	8.98

This table shows that there was no significant difference in percentage of tumors found between the two groups of birds. Some of the tumors found in the apparently normal birds were probably early stages of tumors which might later have caused the death of the individual affected. A study of the individual cases of birds with tumors (see Table IV) shows that while in several cases the tumors were the probable cause of death, yet there were many others among the birds which died from natural causes in which the cause of death was entirely unrelated to the presence of the tumor. The close agreement of the two groups in percentage of birds with tumors strengthens the conclusion that in this flock at least there are about 90 cases of tumors per 1,000 birds.

In order to study the influence of age and sex upon the occurrence of tumors, age-frequency distributions were made for each sex. The birds were grouped into half-year classes. There were a few birds whose exact age was not known. These could be classified as "young" (under 2 years) or "old" (over 2 years). The percentage of the birds of each age group which had tumors was then calculated separately for each sex and for the two sexes together. These data are given in Table II.

This table shows that of the 880 birds only 44 were males, while 836 were females. This difference is due merely to the fact that in the adult flocks only a few males were kept (for breeding purposes) and a great many females. It indicates nothing as to the relative morbidity of males and females. Considering the small number of males, it is possible that the apparent difference in the sexes in regard to the occurrence of tumors, 6.82 per cent in the males and 9.09 per cent in the females, may not be significant. A study of the individual cases, however (see Table IV), shows that the organs most frequently affected in the females are the genital organs. It may easily be that on this account there is a real difference in the sexes.

A study of Table II shows that there is a significant correlation between age and the percentage of birds which have tumors. This is also shown in Table III, which is a summary of the data in Table II, combining the

data on all the birds, whether or not their exact ages were known, into two classes, young (under $2\frac{1}{4}$ years) and old (over $2\frac{1}{4}$ years).

TABLE II.—*Relation of age and sex to the occurrence of tumors in the domestic fowl*

Age in years (mid-points of class).	Females.				Males.				Males and females.			
	Number with tumors.	Number without tumors.	Total number.	Percentage with tumors.	Number with tumors.	Number without tumors.	Total number.	Percentage with tumors.	Number with tumors.	Number without tumors.	Total number.	Percentage with tumors.
$\frac{1}{2}$	5	81	86	5.81	0	4	4	0	5	85	90	5.56
1.....	39	424	463	8.42	1	24	25	4.00	40	448	488	8.20
$1\frac{1}{2}$	5	105	110	4.55	1	3	4	25.00	6	108	114	5.26
2.....	4	60	64	6.25	0	1	1	0	4	61	65	6.15
Total, $\frac{1}{4}$ to $2\frac{1}{4}$ years.....	53	670	723	7.33	2	32	34	5.88	55	702	757	7.27
$2\frac{1}{2}$	3	22	25	12.00	0	0	0	0	3	22	25	12.00
3.....	5	18	23	21.74	0	1	1	0	5	19	24	20.83
$3\frac{1}{2}$	1	1	2	50.00	0	0	0	0	1	1	2	50.00
4.....	0	1	1	0	0	1	1	0	0	2	2	0
$4\frac{1}{2}$	1	0	1	100.00	0	0	0	0	1	0	1	100.00
5.....	0	2	2	0	0	0	0	0	0	2	2	0
$5\frac{1}{2}$	0	0	0	0	0	1	1	0	0	1	1	0
6.....	1	0	1	100.00	0	1	1	0	1	1	2	50.00
Total, $2\frac{1}{4}$ to $6\frac{1}{4}$ years.....	11	44	55	20.00	0	4	4	0	11	48	59	18.64
Total, $\frac{1}{4}$ to $6\frac{1}{4}$ years.....	64	714	778	8.23	2	36	38	5.26	66	750	816	8.09
Exact age unknown:												
Young.....	1	0	1	100.00	0	2	2	0	1	2	3	33.33
Old.....	11	46	57	19.30	1	3	4	25.00	12	49	61	19.67
Total.....	76	760	836	9.09	3	41	44	6.82	79	801	880	8.98

TABLE III.—*Summary of the data showing the relation of age and sex to the occurrence of tumors in the domestic fowl*

Age.	Females.		Males.		Males and females.	
	Total number.	Percentage with tumors.	Total number.	Percentage with tumors.	Total number.	Percentage with tumors.
Young ($\frac{1}{4}$ to $2\frac{1}{4}$ years).....	724	7.46	38	5.56	760	7.37
Old ($2\frac{1}{4}$ to $6\frac{1}{4}$ years).....	112	19.64	8	12.50	120	19.17
Total.....	836	9.09	44	6.82	880	8.98

This table shows that while only 7.46 per cent of the females under $2\frac{1}{4}$ years have tumors, 19.64 per cent of those over $2\frac{1}{4}$ years are affected. The result for the males agrees essentially with that for the females, but the number of males is too small to allow us to consider this result as necessarily significant. It is, however, quite certain that the probability of the presence of a tumor in a bird increases as the bird grows older.

The records available for this study show in which organs the tumor is located and whether it is of cystic or solid-tissue structure. These data are given in Table IV.

TABLE IV.—Data on all the cases of tumors which have been observed at the poultry plant of the Maine Experiment Station, giving their structure and the organs in which they were located—Continued

Autopsy No.	Bird No.	Age.	Sex.	Kind of tumor.	Tumors ^a located in or attached to—												Cause of death.			
					Ovary.	Oviduct wall.	Oviduct ligament.	Mesentery.	Abdominal wall.	Intestine wall.	Kidney.	Gizzard.	Liver.	Spleen.	Pancreas.	Heart.		Testis.	Breast bone.	Organ not recorded.
83.	130B.	Yr. m.		Tissue	+															Killed for data.
114.	1074.	3 0	♀	do.																Congestion of lungs.
167.	1067.	3 1	♀	do.		+														Do.
20H.	908.	3 5	♀	Cystic.	++					++										Roup.
1229.	9024.	4 7	♀	do.	+++															Killed because of roup.
952.	9024.	5 10	♀	do.	+++															Peritonitis due to egg masses in body cavity
906.	9024.	5 10	♀	do.																Unknown.
973.	000.	Young.		do.	+															Do.
185.	13A.	Old.	♀	Tissue	+															Do.
181.	98.	Old.	♀	do.	+															Do.
187.	349.	Old.	♀	do.				++												Killed for data.
45.	349.	Old.	♀	do.	+															Do.
80.	395.	Old.	♀	do.	++															Do.
68.	395.	Old.	♀	do.	++															Do.
58.	30.	Old.	♀	do.	++															Do.
80.	150.	Old.	♀	Cystic.	+++															Do.
65.	152.	Old.	♀	do.	+++															Do.
94.	2.	Old.	♀	Tissue	+															Do.
93.	93.	Old.	♀	do.	+															Do.
954.	954.	Old.	♀	do.	+															Probably tumor.
Total.	79.		76 ♀ 13 ♂		37	10	8	8	13	5	5	1	2	2	2	1	1	1	2	98 tumors.
Total percentage ^b					37.76	10.20	8.16	8.16	13.27	5.10	5.10	1.02	2.04	2.04	2.04	1.02	1.02	1.02	2.04	

^a Asterisk (*), organ hypertrophied probably by infiltration with tumor cells.^b Percentages are calculated on base of 98 tumors, although they all occurred in 79 birds.

Attention has already been called to the fact that tumors occurred as frequently in apparently normal birds which were killed as in those which died from natural causes. From the data given in Table IV it may be seen that many of the birds with tumors died from diseased conditions apparently not related to the presence of the tumors. There were, however, a number of cases where the size and distribution of the tumors and the condition of the organs to which they were attached indicated that the tumors were the probable cause of death. Associated with many cases of tumors was a hypertrophied condition of the liver, spleen, or kidneys. The liver was most often affected. In fact, 19, or 24.05 per cent, of the individuals having tumors had enlarged and soft, friable livers. In the absence of microscopic examination of these organs, it can not be definitely stated that this hypertrophy was due to infiltration with tumor cells.

Table IV also shows that in several cases the immediate cause of death was internal hemorrhage, either from the tumor surface, the tissue immediately beneath, or the hypertrophied liver or spleen. There were several tumor cases in which death was recorded as due to internal hemorrhage but in which the bleeding point was not recorded. It is probable that in these cases also the bleeding took place either from the tumor or from the hypertrophied liver or spleen.

Our macroscopic examination of the tumors limited their classification to the two groups of tissue tumors, formed of solid masses of tissue or sometimes of large tissue masses inclosing masses of pus, mucus, or clotted blood, and cystic tumors, which were sacs filled with liquid. Table IV shows that 18, or 22.78 per cent, of the tumors observed were cystic, while 59, or 74.68 per cent, were tissue tumors. There were two cases (2.59 per cent) of ovarian tumors where cysts were attached to tissue tumors.

Table IV also shows the organ distribution of the tumors. It should be borne in mind that this is essentially the distribution in females, as only three males are included in the data. The organ most frequently affected is the ovary (37.76 per cent¹ of all the tumors occur in that organ). The oviduct wall and ligament harbored 18.36 per cent—that is, in the female the genital organs are the organs most frequently affected by tumors. The number and percentages for each of the other organs are given in the table. Table IV also shows that in most cases the tumor was confined to one organ. In 15 cases, however, the tumor had undergone metastasis, since tumors of similar sorts occurred in 2 (11 cases), 3 (3 cases), or 4 (1 case) organs. Attention has already been called to the frequent association of hypertrophied livers, spleens, and kidneys with defined tumors in other organs.

¹ These percentages are calculated on the basis of 98 tumors, although they all occurred in 79 individuals

SUMMARY

The purpose of the present paper is to record the data on the frequency of occurrence of tumors in the domestic fowl which have been collected during eight years' routine autopsy work at the Maine Agricultural Experiment Station.

The chief points brought out by an analysis of these data are as follows:

(1) Of the 880 birds autopsied 79, or 8.96 per cent, had tumors. That is, there were 90 cases of tumors per 1,000 birds.

(2) There was no significant difference in frequency of occurrence of tumors between birds which died from natural causes and apparently normal birds which were killed.

(3) There is a significant positive correlation between age and the occurrence of tumors. Only 7.37 per cent of the birds under $2\frac{1}{4}$ years had tumors, while neoplasms were present in 19.17 per cent of those that were over that age.

(4) In birds with tumors which died from natural causes, the tumors were directly or indirectly the probable cause of death in from one-third to one-half the cases.

(5) There was a decided tendency for the association of hypertrophied (apparently due to cell infiltration) liver, spleen, or kidney with the presence of tumors in other organs.

(6) Death often resulted from internal hemorrhage from the tumor, the underlying tissue, or the hypertrophied liver or spleen.

(7) The tumors can be classified into cystic and tissue tumors; 22.78 per cent of the tumors were of cystic and 74.68 per cent of solid-tissue structure. There were two cases of tissue tumors to which cysts were attached.

(8) In the females¹ the organs most frequently affected were the genital organs; 37.76 per cent of all the tumors being in the ovary and 18.36 per cent in the oviduct and oviduct ligament.

(9) In most cases the tumors were confined to one organ. In 15 cases, however, the tumor had evidently undergone metastasis, since tumors of similar nature occurred in from two to four organs.

¹ Autopsies were made on too few males to yield reliable data.

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Dean, Agricultural Experiment Station of
the University of Minnesota*

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

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INHERITANCE OF LENGTH OF POD IN CERTAIN CROSSES

By JOHN BELLING,¹

Assistant Botanist, Florida Agricultural Experiment Station

INTRODUCTION

The inheritance of a difference between two plants has sometimes, though not often, been studied both qualitatively and quantitatively. Correns (5)² has shown that this can be done even with differences in flower color. The inheritance of a large-size difference can occasionally be followed by mere inspection, as in crosses of some tall and dwarf races of peas (*Pisum sativum*) (13); sweet-peas (*Lathyrus odoratus*) (1, p. 280-281); beans (*Phaseolus vulgaris*) (8); and maize (*Zea mays*) (10).

Even with accurate measurements, however, it will probably not be possible to keep track of a single small-size difference, for its segregation may be masked by the modifications. But if several small genetic differences affect the size of the same plant organ, it would usually be still less possible to disentangle the segregation in the second generation of a cross, as Johannsen (12) has proved. The masking effect of the modifications may, however, be lessened by choosing those plant organs which are least liable to modification and which are also repeated many times on each plant, such as flowers (6) or pods with the modal number of consecutive ripe seeds (2). In one such case some of the members of a fraternity were grown on poles 8 feet apart, and others were sown at intervals of 4 feet in a thick row of sorghum. Though the crops of the stunted plants averaged only one-twentieth of those of the others, yet the average length of their 5-seeded pods reached 94 per cent of that of the pods of the well-nourished plants.

In the reciprocal crosses described in this paper, the length of pod was first studied qualitatively and then quantitatively. All the families

¹ I express my thanks to Messrs. C. D. Gunn and C. W. Long, of the Florida Experiment Station, for their careful work in measuring pods.

² Reference is made by number to "Literature cited," pp. 419-420.

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grown were selected with the aim of obtaining useful agricultural plants. A fairly complete third generation was raised, but the fourth generation was the result of selection and was the opposite of a random sample.

QUALITATIVE INVESTIGATION

The Florida velvet bean (*Stizolobium deeringianum*) was crossed both ways with the Philippine Lyon bean (*S. niveum*). A pertinent description of these plants has been given in my account of the inheritance of semisterility (4). The Florida velvet bean has a short pod (Pl. XL, fig. B), while the pod of the Lyon bean (Pl. XL, fig. C) is about half as long again and is broader. The pods of the first-generation hybrid plants were as long as, or slightly longer than, those of the Lyon. The progeny of the hybrids in the second generation could be divided by inspection into short-podded plants and long-podded plants. The short pods could be identified, even when young, by their greater proportional width. Although both short pods and long pods varied greatly in size on different second-generation plants, yet no case was met with where the classification could not be carried out when all the pods on a plant were taken into account. Plate XL, figures A and D, shows typical pods of second-generation plants with pods shorter than the Florida velvet bean and longer than the Lyon bean pods. The difference between short and long pods was sharply marked in all the segregating third-generation families.

Tables I, II, and III give the results of field inspection, checked by examination of the pods after harvesting.

TABLE I.—Length of pods in first-generation bean crosses

Parentage. ^a	Number of plants with—	
	Long pods.	Short pods.
Florida velvet bean × Lyon bean.....	7
Lyon bean × Florida velvet bean.....	6
Total.....	13

^a The pollen parent is given last throughout this article.

TABLE II.—Length of pods in second-generation bean crosses

Parentage.	Progeny ratio.		Calculated ratio.		Deviation.	Probable deviation.
Florida velvet bean × Lyon bean.....	Long.	Short.	Long.	Short.		
	140	: 49	141.75	: 47.25	—1.75	4.0
Lyon bean × Florida velvet bean.....	375	: 120	371.25	: 123.75	+3.75	6.5
Total.....	515	: 169	513	: 171	+2.0	7.6

The most probable single ratios have been calculated on the hypothesis that there are three chances for the long pod to one chance for the short pod. However, by the theory of probability, a deviation from the whole numbers nearest to these calculated ratios is far more likely to occur than not. The most probable deviation has been calculated by the conventional formula,¹ and is given in the last column of Table II. Since the actual are not greater than the calculated deviations, it is probable that there is no interference with the random segregation of the long and the short pod, with three chances for the long to one chance for the short pod.

The third-generation families of the Florida velvet bean \times Lyon bean were grown in an elimination field among crowding sorghum, where there was some selective elimination of short-podded plants (3). Hence the ratios are useless here. Two long-podded parents, however, of those whose families were grown on poles gave a total of 49 long-podded to 13 short-podded (calculated, $46.5 \pm 2.3 : 15.5 \mp 2.3$). In the third generation of the Lyon bean \times Florida velvet bean, 17 families of more than 8 members each from long-podded parents were grown on poles. The totals of the 11 segregating families among these amounted to 231 long-podded and 76 short-podded plants, the calculated nearest whole numbers being 230 and 77. The long-podded homozygotes could not be distinguished by inspection from the heterozygotes. These results are given in Table III. The abbreviations used in this and the subsequent tables in this paper are "V" for Florida velvet bean and "L" for the Lyon bean.

TABLE III.—Length of pods in third-generation bean crosses from long-podded parents

Parentage.	Progeny ratio.		Calculated ratio.		Deviation.	Probable deviation.
	Long.	Short.	Long.	Short.		
LV-92.....	23	: 0
LV-548.....	30	: 0
LV-569.....	38	: 0
LV-558.....	20	: 0
LV-27.....	28	: 0
LV-311.....	9	: 0
LV-80.....	25	: 12	27.75	: 9.25	-2.75	± 1.8
LV-113.....	22	: 6	21	: 7	+1.0	± 1.5
LV-279.....	24	: 6	22.5	: 7.5	+1.5	± 1.6
LV-486.....	31	: 7	28.5	: 9.5	+2.5	± 1.8
LV-91.....	21	: 4	18.75	: 6.25	+2.25	± 1.5
LV-114.....	13	: 4	12.75	: 4.25	+0.25	± 1.2
LV-310.....	26	: 8	25.5	: 8.5	+0.5	± 1.7
LV-468.....	15	: 10	18.75	: 6.25	-3.75	± 1.5
LV-527.....	15	: 8	17.25	: 5.75	-2.25	± 1.4
LV-461.....	28	: 8	27	: 9	+1.0	± 1.8
LV-392.....	11	: 3	10.5	: 3.5	+0.5	± 1.1
Total.....	231	: 76	230.25	: 76.75	+0.75	± 5.1

¹ I have used the ordinary formula for probable deviation, which, however, does not seem to be appropriate (except with large numbers) to any but a 1 to 1 segregation. East and Hayes's practical test of this formula with large numbers (7) shows that it will in that case fit a 3 to 1 segregation with sufficient accuracy. Hence, the calculated probable deviations in Table III, where the numbers are small, are not reliable.

Out of these 11 segregating families, 5 show proportions with a greater deviation than the probable and 6 have a less deviation. The chances for deviations above and below the probable are theoretically equal. The greatest deviation is less than three times the probable. In 3 of the families the calculated numbers occur, since fractions of plants are impossible. Of the other families 5 show an excess of long-podded and 3 an excess of short-podded plants. Hence, the ratios for the third generation conform closely to the theory of probability. However, a further test can be made. It seems that a perfectly random distribution, with three chances for long pods to one chance for short pods, should give for any number of equal groups of n plants each a frequency distribution of numbers of long-podded plants in the groups in classes from n to 0 which corresponds to the terms of the binomial $(3 + 1)^n$. If all the segregating families of the third generation are divided into 76 consecutive groups of 4 plants each in the same order as grown in the field, omitting the last 3 plants out of the total of 307, we have the groups as given in Table IV.

TABLE IV.—Third-generation segregating families in groups of four plants

Pods.		Groups.		Deviations.
		Found.	Calculated.	
<i>Long.</i>	<i>Short.</i>			
4	: 0	27	24	+3
3	: 1	27	32	-5
2	: 2	18	16	+2
1	: 3	4	4	0
0	: 4	0	0	0

There is, thus, a fair agreement of the actual figures with those calculated for a random distribution with three chances for long to one chance for short pods.

Of the random sample of 17 families from long-podded parents given in Table III, 11 families segregated into long podded and short podded, while 6 families were constantly long podded. The calculated nearest whole numbers are also 11 and 6.

Eleven second-generation short-podded plants gave only short-podded progeny. One of these has been grown to the fifth generation, giving only short-podded progeny. Four second-generation long-podded plants which were constant in the third generation have been grown to the sixth generation on a field scale without throwing any short-podded progeny.

Therefore, the whole of the second-generation plants were probably in the proportion of 1 constant short-podded to 1 constant long-podded to 2 heterozygous long-podded plants.

Now, we must assume, with Mendel, Correns, and Bateson, that this difference of long-podded and short-podded plants corresponds to a difference between the pollen grains and egg cells of the Florida velvet

bean, on the one hand, and those of the Lyon bean, on the other. But, according to the special investigations of Strasburger and his coworkers, only a sperm nucleus without cytoplasm passes from the pollen tube to the egg cell in most angiosperms. If this is the case here, the progeny of the Florida velvet bean \times Lyon bean receives cytoplasm only from the Florida parent; and the progeny of the reciprocal cross has cytoplasm only from the Lyon bean. Hence, the genetic difference which determines the visible difference between long and short pods is a difference of the nuclei, not a difference of the cytoplasm. If we call this particular nuclear difference of the gametes, $E-e$, the nuclear difference of the zygotes (the Florida velvet bean and the Lyon bean plants) will be $E_2 - e_2$. ($E_2 = E + E$.) Since we have no definite base of measurement, it is useful in many cases to take the recessive as our base and to regard e as zero. This is merely a convention.

To sum up, the Florida velvet bean and the Lyon bean have one main genetic difference affecting pod length. This genetic difference segregates in typical Mendelian fashion.

QUANTITATIVE INVESTIGATION

Investigators of the inheritance of differences in size have found that in many cases these differences are inherited as if several genetic differences (factors) were concerned and dominance was lacking. For instance, in East's masterly investigation of the inheritance of flower size in crosses of two species of *Nicotiana* (6), the first-generation mean flower length was near the geometrical mean of the parent flower lengths, while the second-generation mean was only slightly greater. The frequency array of the flower lengths of the second-generation plants formed a continuous series between the two grandparental means, with the mode below the center. If dominance had been present, the second-generation mean would have been less than the first-generation mean and the first-generation mean should have approached that of the long-flowered parent (supposing all factors were positive). Emerson (9) obtained similar results from a cross of short and long squashes (*Cucurbita pepo*). Groth (11) in many crosses of tomato (*Lycopersicon esculentum*) found the first-generation fruit length near the geometrical mean of the parent lengths. However, the strict proof of this absence of dominance demands, I think, the isolation of a family in which only one such genetic difference is segregating.

The hypothesis that size factors act as multipliers was, I believe, first applied by East (6). Groth's results are readily explicable on this hypothesis. A similar assumption has been made by Punnett and Bailey (14).

To sum up, previous work favors the hypothesis that some size factors show no dominance and act as multipliers.

PARENT PLANTS

In 1910 the mean of the averages of all the ripe 5-seeded pods on 11 plants of the Florida velvet bean (pedigreed line) was 62.9 mm. The mean of the average lengths of the 5-seeded pods of 9 plants of the Lyon bean (pedigreed line) was 92.7 mm. Some of these Lyon bean plants grew in a sandy spot and were stunted; hence the calculated mean is probably too low.

In 1912 the mean of the averages of all the 5-seeded pods of 2 pedigreed Florida velvet bean plants was 62.8 mm. and that of 2 pedigreed Lyon bean plants was 94.5 mm. These plants were grown on poles and were kept free from caterpillars. From 4 more Florida velvet bean and 42 more Lyon bean plants, of the same families, large samples were picked, and all the 5-seeded pods in these samples were measured, but in picking such samples the conspicuous best racemes are probably picked first, and the averages (63.2 and 95.6), which include these samples, are probably too high.

To sum up, the most reliable measurement of the average length of the dry 5-seeded pods of the pedigreed line of the Florida velvet bean was probably 62.8 mm. and that of the Lyon bean 94.5 mm.

FIRST GENERATION

The 5-seeded pods of the 7 first-generation plants were not separately measured in 1909, although many pods were measured. The measurements of 883 seeds from all parts of the pod gave an average of 15.5 mm. The measurements of 613 seeds of the Lyon bean from all parts of the pod gave an average of 15.1 mm. The excess of the first-generation seed length over that of the Lyon bean is in part, or wholly, due to the many gaps in the seed rows of the semisterile first-generation plants. These gaps permit the rounding off of the ends of the seeds, whereas the Lyon bean seeds are usually flattened at the ends by mutual pressure. For five seeds, the maximum excess of the hybrids over the Lyon bean thus is 2 mm.

In 1911 the six first-generation plants were more or less frosted. Only three 5-seeded pods were measured, averaging 98 mm.

To sum up, the average length of the 5-seeded pods of the first-generation plants is probably less than 2 mm. above that of the Lyon bean.

SECOND GENERATION

In Table V are given the frequency arrays of the average lengths of the ripe 5-seeded pods of the plants with white shoots of the second generations of the reciprocal crosses. The plants with black shoots (three-sixteenths of the whole) are not included, because they usually either bore no pods or bore few pods on large plants and so had their pod length physiologically increased. A trial showed that when all

young pods except eight were removed from a plant of the Florida velvet bean the length of 5-seeded pods increased from 63 to 73 mm. The plants in 1912 were grown in an especially favorable season, and more of the late plants had time to ripen their pods than in 1910.

TABLE V.—Frequency arrays of the average lengths of ripe 5-seeded pods of bean plants with white shoots of second generations of the reciprocal crosses (classes of 3 mm.)

Length of pod, mm.....	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	97	100	103	106	109	112	115	Average lengths.	Total number of plants.
Florida velvet bean.....					6	4	1																<i>Mm.</i> 62.9	11
Lyon bean.....														1	5	2	1						92.7	9
F ₁ hybrids.....																x							98?
F ₂ hybrids.....	1	4	8	10	9	8	4	1	1		8	17	9	23	25	11	13	10	7	4	2		62.7 and 94.2	46 and 129
F ₂ parents.....			(1)	(1)							(1)	(2)	(1)	(2)	(3)	(2)	(2)	(6)	(2)	(1)	(1)	a(1)

[illegible]

^a Black plant.

The actual averages ¹ were:

	Short pods.	Long pods.
1910.....	62.7	94.2
1912.....	62.7	94.7

These are sensibly the same as the most trustworthy averages (62.8 and 94.5 mm.) for the Florida velvet bean and the Lyon bean in 1912.

The average of the first-generation plants is probably near 95 mm. The average of the long-podded plants of the second generation is 94.7 mm. Therefore, the factor *E* is probably completely dominant.

Thus, in the second generation the short pods and the long pods give the grandparental averages. The minor factors affecting pod length have not perceptibly altered the averages by their segregation, which agrees with the conclusion that *E* was completely dominant and the minor factors showed zero dominance and acted symmetrically with regard to both long and short pod, decreasing and increasing to the same extent each parental pod length. Calculation shows in this case that the increase of the second-generation averages over the parental lengths, which is a consequence of the hypothesis that the factors act as multipliers, is so small as to be negligible.

¹ The averages have been calculated from the actual figures, not from the frequency classes.

Dividing the second-generation variates into groups on each side of the means, we have:

Year.	Number of short pods.		Number of long pods.		Differences.
	Below mean.	Above mean.	Below mean.	Above mean.	
1910.....	24	22	71	58	2 and 13
1912.....	51	49	165	150	2 and 15

In each case there are fewer variates above than below the mean. This agrees with the hypothesis that the factors act as multipliers.

The second-generation means, including both short and long, were 85.9 and 86.9 mm. These two determinations average 86.4 mm. If E is completely dominant and the minor factors act symmetrically, the second-generation mean will be $\frac{1}{4}(62.8 + 3 \times 94.5) = 86.6$. This is sensibly the same as the actual average, 86.4.

If factor E is a multiplier and completely dominant, we may find its multiplying value in several ways:

Parents—

1910..Lyon bean+Florida velvet bean= $92.7 \div 62.9 = 1.47$. (Lyon bean value is too low.)

1912..Lyon bean+Florida velvet bean= $94.5 \div 62.8 = 1.50$. (Two plants each.)

1912..Lyon bean+Florida velvet bean= $95.6 \div 63.2 = 1.51$. (Including samples.)

Second generation—

1910..Long+short= $94.2 \div 62.7 = 1.50$.

1912..Long+short= $94.7 \div 62.7 = 1.51$.

This gives 1.50 to 1.51 for the multiplying value of Ee or E_2 compared with e_2 .

The extremes of the two crosses were:

	Short pods.	Long pods.
1910.....	52 and 76	81 and 113
1912.....	53 and 75	79 and 113

The results in the third and fourth generations show that these extreme values are inherited. The values of 1912 are probably the more reliable. If E is completely dominant and the factors are multipliers, the multiplying value of E is given by:

Shortest long pod+shortest short pod = $79 \div 53 = 1.49$

Longest long pod+longest short pod = $113 \div 75 = 1.51$

If E had shown incomplete dominance, the second value should have been markedly greater than the first. The average multiplying value of Ee or E_2 is here 1.50.

The square root of the product of the extremes should give the means nearly and the grandparental means more nearly.

$$\begin{array}{lll} \sqrt{53 \times 75} = 63.0 & \text{Mean} = 62.7 & \text{Grandparental mean} = 62.8 \\ \sqrt{79 \times 113} = 94.5 & \text{Mean} = 94.7 & \text{Grandparental mean} = 94.5 \end{array}$$

Lastly the combined multiplying value of all the minor factors (when double) is given thus:

Quotient of extremes of short-podded plants. 75 ÷ 53 = 1.42
 Quotient of extremes of long-podded plants. 113 ÷ 79 = 1.43

The standard deviation in the second generation was:

	Short pods.	Long pods.
1910.....	5.1	7.4
1912.....	5.2	6.8

That the standard deviation of the long-podded is greater than that of the short-podded plants is in agreement with the hypothesis that the minor factors act as multipliers. If *E* is completely dominant, there is no difference in the action of *Ee* and *E₂* to increase the standard deviation of the long-podded plants. The ratios of the two standard deviations in each of the two crosses (1.4 and 1.3) are not quite 1.5, as theory would seem to demand if all the variation were genetic. (See, however, below.)

The coefficients of variation were:

	Short pods.	Long pods.
1910.....	8.2	7.8
1912.....	8.3	7.2

If the variation were purely genetic, these coefficients should, I think, be nearly equal. East (6), however, gives the variation coefficient of the corolla-tube lengths of two parent lines of *Nicotiana* spp. as 8.9 for the short-flowered (170) plants and 6.8 for the long-flowered (167) plants. This variation was presumably not genetic. Judging from this, any modifications would tend to increase the coefficient of variation of the short-podded more than that of the long-podded plants. Hence, it is possible that the slight lowering of the standard deviation of the long-podded plants from the theoretical 1.5 to 1.4, or 1.3 times that of the short-podded plants, is an effect of modifications. Hence, this result does not, I think, disagree with the hypothesis that the factors act as multipliers.

That neither short-podded nor long-podded second-generation plants show a significant increase in either range or standard deviation by more than doubling their number seems to indicate that the genetic series can be fully developed with about 50 plants. But the absence of linkage has not been proved, and until this has been done no definite deductions as to the number of minor factors can be made.

The ranges are:

	Short pods. Mm.	Long pods. Mm.	Ratio of long to short pods.
1910.....	24	32	1.33
1912.....	22	34	1.55

On the hypothesis of factors acting as multipliers, the range of the long-podded plants should be about 1.5 times that of the short-podded plants, as it is in the more reliable 1912 results.

To sum up, the results of investigation of the second generations agree with the hypotheses that all the factors act as multipliers; that factor *E* is completely dominant; that the minor factors show zero dominance; that the minor factors act symmetrically with regard to each of the two grandparental lengths, which is not the case in a cross of the Florida velvet bean by the Yokohama bean (*Stizolobium hassjoo*).

THIRD GENERATION

Table VI gives all the third-generation families, grown in the elimination field, which segregated measurable short podded; and also all which did not, but had eight or more measurable survivors. Because of the crowding, these results are not so reliable as those given in Table VII, which include all the families grown on poles in 1913.

TABLE VI.—Frequency arrays of the average lengths of ripe pods of the third generation
Florida velvet bean \times Lyon bean (classes of 3 mm.)

[The asterisk (*) shows the pod length of the parent plant of the family.]

Parentage.	Progeny.																								Average length of pod.		
Length of pod, mm.	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	97	100	103	106	109	112	115	118	121	Mm.	
VL-292	1	1						1	1			3	3	*3			1										.84
VL-133	1		1	1	1			1				2	3	*5		3	2		*2								.88
VL-171			1	1	1					1				1	1	1	2										.91
VL-325				1									1			*1	1			1							.92
VL-88					1	1	1	2	1			1		1		1	3	1	1		2	1					.93
VL-509					1	1	1	2	1				1		1		1				2	1		1			.102
VL-164					1	1	1										2	*3	6		6	4		1	1	1	.103
VL-294								1																			(.107)
VL-85								1	1						1		1	1	1	3	*3	2	2				.104
VL-158	1	2	3	*4																							.55
VL-569				2	*2	3	1	2																			.64
VL-319									1	1	2	3	2														.83
VL-147										1	1	2	3														.86
VL-114											1	6	2	3		*1	3										.89
VL-255											1	1	2	3		3	*1										.90
VL-92											1	1			*1	3	1			1							.92
VL-94											1					1	1	2		*2							.97
VL-194												2		1	2	2			*1	3							.99
VL-610															1	*1	1	3	3	1							.99
VL-102															2	3	4	4		*	1	1	2				.99
VL-120															1	2		1	*2		2			1			.100
VL-177																	2	3		1	*	1					.101
VL-251														1		1	1			1	4	*2					.101
VL-515 ^b																1	4	3	*10	6	2						.102
VL-297 ^b														1	1		1	6	2	2	2		*	1	1		.102
VL-480																	1	3		2	3		*2				.105
VL-515 ^c																			1		*2	8	5	5	2	1	.109
VL-297 ^c																			1	1	1	1	*0	2	2		.110

^a The averages for the first nine families refer to the long-podded plants alone.

^b Grown in the elimination field in 1911.

^c Grown on poles in 1912.

TABLE VII.—Frequency arrays of the average length of ripe pods of the third-generation Lyon bean \times Florida velvet bean (classes of 3 mm.)

(The asterisk (*) indicates the pod length of the parent of the family)

F ₂ parentage.	F ₃ progeny.																							Average length of pod.	
Length of pod.....mm.	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	97	100	103	106	109	112	115		
																									Mm.
LV-113.....	3	1	—	1	—	—	—	—	—	5	*5	3	4	—	—	—	—	—	—	—	—	—	—	—	.80
LV-279.....	1	—	1	—	1	—	—	—	—	5	3	6	2	—	—	1	—	—	—	—	—	—	—	—	.81
LV-468.....	1	—	1	—	3	—	—	—	—	—	—	4	—	1	—	3	—	2	—	—	—	—	—	—	.90
LV-461.....	2	—	—	1	—	—	—	—	—	2	7	4	1	—	5	2	—	1	—	—	—	—	—	—	.90
LV-310.....	2	—	—	—	—	1	—	2	3	—	—	—	—	3	3	3	2	—	2	1	—	1	—	—	.95
LV-80.....	—	—	3	3	1	—	—	—	—	—	—	—	—	—	7	2	3	1	1	—	—	1	—	—	.96
LV-527.....	—	2	1	—	—	2	—	—	—	—	—	1	—	3	1	—	—	2	1	—	2	—	—	—	.96
LV-486.....	1	—	1	3	2	—	—	—	—	—	—	2	1	—	5	6	5	5	1	2	—	1	—	—	.96
LV-114.....	—	—	—	—	1	1	—	—	—	—	—	—	—	—	1	2	2	1	—	—	—	1	—	—	.99
LV-91.....	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	2	2	1	—	6	—	1	—	1	.101
LV-92.....	—	—	—	—	—	—	—	—	—	—	—	1	—	3	1	—	5	3	—	1	—	—	—	—	.92
LV-558.....	—	—	—	—	—	—	—	—	—	—	—	1	—	2	4	4	4	1	1	—	1	—	—	—	.95
LV-27.....	—	—	—	—	—	—	—	—	—	—	—	1	—	1	2	5	5	5	5	—	—	1	—	—	.98
LV-548.....	—	—	—	—	—	—	—	—	—	—	—	1	—	2	3	3	3	5	1	—	—	—	1	—	.99
LV-569.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	4	3	4	2	3	2	—	—	—	.99

In length of pods, VL-319 and LV-113 are the two lowest families from long-podded parents. The family of VL-319 ranges from 76 to 88 mm. and seems homozygous for *E*; that of LV-113 ranges from 76 to 85 mm., and throws short-podded, ranging from 49 to 58 mm. The parental lengths were 82 and 79 mm., respectively. To all appearances these two families are homozygous recessives for minor factors (regarded as positive).

VL-480 and VL-85 are the two highest families with the highest averages. (VL-297 was a nearly normal black plant throwing velvet.) The family of VL-480 ranges from 97 to 112 mm. and is homozygous for *E*. VL-85 ranges from 88 or 94 to 112 and throws short-podded of 70 to 73 mm. long. The parental lengths were 113 and 106 mm., respectively. VL-480, as shown in the fourth generation, is apparently homozygous for all minor factors, as well as for *E*.

Thus, both near the minimum and near the maximum of the second-generation long-podded plants, we find plants homozygous and heterozygous for *E*. Hence, *E* is probably completely dominant.

The numbers in each family are not large enough to determine the separate ranges. The fifth and last lines of Table V show the pod lengths of the parents of these families. The correlation between the average pod lengths of the long-podded parents and the averages of the long-podded plants of their progenies is 82 ± 5 per cent for 36 third-generation families.

The range of the short-podded plants in the various families is from 49 or 52 to 73 mm., and that of the long-podded from 73 to 118 mm. in the elimination field (omitting the black plant, VL-297) and from 76 to 115

mm. for the plants grown on poles. These ranges do not seem to differ significantly from the second-generation ranges.

The families are arranged according to the means of their long-podded plants. LV-310, exceptionally, as was marked in the field, throws short-podded plants with pods unusually long in comparison with those of its long-podded progeny. Whether this is a genuine exception can only be determined by growing further generations from it. This is being done.

In Table VIII the averages of the short-podded plants in each family are compared with the averages of the long-podded plants in the same families. If *E* is completely dominant and none of the minor factors show linkage (coupling or repulsion) with *E*, then the average ratio of the pod length of long-podded to short-podded plants should be about 1.5 in each family. With the exception of the family of LV-310, the ratio comes as close to 1.5 as can be expected in small families, averaging 1.52.

TABLE VIII.—Comparison of the length of pods of the short-podded plants in each family with those of the long-podded plants in the same families. Third generation. Parents heterozygous for *E*

Parentage.	Pod length of parent.	Pod length of progeny.		Ratio of lengths.	Difference from parent.
		Average of short-podded plants.	Average of long-podded plants.		
	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>		
LV-113.....	79	51.2	80.3	1.57	+ 1
LV-279.....	88	57.3	81.1	1.42	- 7
VL-292.....	88	53.0	83.8	1.58	- 4
VL-133.....	86	60.2	87.8	1.46	+ 2
LV-401.....	94	56.0	89.6	1.60	- 6
LV-468.....	92	58.4	89.7	1.54	- 2
VL-171.....	101	59.8	90.5	1.51	-10
VL-88.....	103	65.8	93.3	1.42	-10
LV-310.....	95	71.0	95.4	1.34	0
LV-80.....	91	60.3	95.5	1.58	- 5
LV-486.....	100	61.1	96.2	1.58	- 4
LV-527.....	95	62.6	96.4	1.54	+ 1
LV-114.....	98	62.5	99.3	1.59	+ 1
VL-509 <i>a</i>	93	65.8	101.6	1.55	<i>a</i> + 9
VL-164.....	98	65.0	102.7	1.58	+ 5
VL-85.....	106	71.0	103.5	1.46	- 2
Average.....				1.52	- 2

a Part of this family was grown on poles.

If the minor factors show zero dominance, the average of the long-podded progeny in each family should equal the parental average, the theoretical excess here being negligible. On the whole, the long-podded plants average 2 mm. shorter than their parents. This is in part due to the stunting in the elimination field, and also possibly to the severe drought in 1913. In both cases the third-generation families were

grown under more adverse conditions than were their second-generation parents.

Table IX compares the parental and progeny pod lengths of families not known to throw short-podded. The averages of the progenies are here less than the parental averages by 3.5 mm. (See above.)

TABLE IX.—Comparison of the pod length of the parents and progeny of families not known to throw short-podded. Third generation. Parents probably or certainly homozygous for *E*

Parentage.	Pod length of parent.	Average pod length of progeny.	Difference from parent.	Parentage.	Pod length of parent.	Average pod length of progeny.	Difference from parent.
	<i>Mm.</i>	<i>Mm.</i>			<i>Mm.</i>	<i>Mm.</i>	
VL-50 ^a	82	83	+1	VL-102.....	102	99	- 3
VL-147 ^a	85	86	+1	VL-194.....	103	99	- 4
VL-114.....	93	89	-4	LV-569.....	104	99	- 5
VL-255.....	97	90	-7	VL-120 ^a	101	100	- 1
VL-92 ^a	92	92	0	VL-177 ^a	105	101	- 4
LV-92.....	96	92	-4	VL-251.....	108	101	- 7
LV-558.....	94	95	+1	VL-515.....	104	102 (109)	- 2 (+5)
VL-94 ^a	104	97	-7	VL-297 ^b	115	102 (110)	-13 (-5)
LV-27.....	103	98	-5	VL-480.....	113	105	- 8
VL-610.....	95	99	+4				
LV-548.....	102	99	-3	Average.....			- 3.5

^a F₂ plants not certainly known to be homozygous for *E*.

^b A black plant throwing velvet.

To sum up, investigation of the third generation gives evidence that *E* is completely dominant; that its multiplying value is 1.5 (one family being an exception); that the genetic range of pod length was fully developed in the second generation; that the minor factors show zero dominance.

FOURTH GENERATION

The frequency arrays of fourth-generation families are given in Table X. By this time it was, of course, known which second-generation plants were *Ee*, and only two *Ee* families were grown. It was not possible to select directly for long-podded plants homozygous for *E*, as selection could only be made after growing the progeny. If the minor factors show zero dominance, selection for specially long pods should be speedily efficacious. Among other desirable characters, extra length of pod was sought for. Hence, the chances were that most selected third-generation plants would be the homozygotes in their families with regard to minor factors.

TABLE X.—Frequency arrays of the average length of ripe pods of fourth-generation crosses of beans (classes of 3 mm.)

* [The asterisk (*) shows the parental pod length]

F ₃ parentage.	F ₄ progeny.																				Average length of pod.
Length of pod...mm.	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	97	100	103	106	109	
VL-10-1.....	1	-4	-2	*1	-2																Mm. .58
LV-486-36.....	1	-3	*3																		.59
LV-486-35.....	3	-1									2	-5	*1	-2	-1						.56 and .89
LV-92-2.....												*	3	-1	-3						.91
LV-92-6.....												1	*11	-8	-1						.89
LV-92-35.....											4	-4	-1	*							.87
LV-92-40.....											2	-6	-3			*					.88
VL-216-1.....											3	-3	-9	-9	*1	-1					.91
LV-558-17.....												*	2	-7	-6	-3					.93
LV-558-24.....												2	-3	*3	-3						.93
LV-558-13.....												1	-1	*3							.92
LV-558-9.....												3	*2		-1						.93
LV-558-11.....												2	-3		*1						.94
LV-569-22.....													5	*3							.92
LV-569-40.....													1	*3	-1						.95
LV-569-6.....													4	-2	*						.92
LV-569-23.....													1	-3	-2	-8	*5				.96
LV-91-16.....														2	-3	-4	*0	-0	-1		.99
LV-91-4.....														4	-0		*3	-4			.102
VL-85-15.....						1									2	-0	-3	*3	-2		.67 and .104
VL-480-6.....																	4	*15	-3	-3	.107
VL-515-21.....															*	4	-5	-6	-4	-2	.105
VL-515-22.....															1	*1	-12	-15	-6	-1	.105
VL-515-23.....																*3	-6	-10	-8	-2	.106
VL-515-35.....																3	*2	-6	-13	-5	.108
VL-515-1.....																3	-1	*0	-1	-3	.108
VL-515-27.....																2	*5	-2	-8	-0	.109
VL-515-31.....																2	-4	-9	*10	-1	.107
VL-297-23.....															*		2	-1	-5	-1	.109
VL-297-19.....																*2	-4	-8	-10	-4	.109
VL-297-5.....																	2	*6	-11	-5	.109
VL-297-11.....																1	-0	-6	-7	-2	.109

One family (from *Ee* parent), LV-486-35, shows a ratio of long-podded mean to short-podded mean of 1.5.

In the families of LV-92, the parents ranged from 82 to 97. The progenies did not sensibly differ. Judging by these, LV-92 was homozygous for minor factors. The same applies to the families of VL-297.

On the other hand, the families of VL-515 showed evidence of the segregation of a minor factor; a segregation also marked in the field.

No indubitable evidence of segregation can be seen in the other fourth-generation families.

In Table XI the pod lengths of the third-generation parents are compared with those of their long-podded progenies. The average of the whole shows an insignificant excess of pod length in the progenies.

TABLE XI.—*Comparison of the pod lengths of third-generation parents with those of their long-podded progeny*

Parentage.	Pod length of parent.	Average pod length of progeny (long-podded).	Difference from parent.	Parentage.	Pod length of parent.	Average pod length of progeny (long-podded).	Difference from parent.
	<i>Mm.</i>	<i>Mm.</i>			<i>Mm.</i>	<i>Mm.</i>	
LV-486-35...	92	89	- 3	LV-91-16...	104	99	- 5
LV-92-2...	81	91	+10	VL-85-15...	105	104	- 1
LV-92-6...	87	89	+ 2	VL-480-6...	105	107	+ 2
LV-92-35...	93	87	- 6	VL-515-21...	98	105	+ 7
LV-92-40...	96	88	- 8	VL-515-22...	99	105	+ 6
VL-216-1...	96	91	- 5	VL-515-23...	101	106	+ 5
LV-558-17...	87	93	+ 6	VL-515-35...	103	108	+ 5
LV-558-24...	94	93	- 1	VL-515-1...	105	108	+ 3
LV-558-9...	94	93	- 1	VL-515-27...	105	109	+ 4
LV-558-13...	95	92	- 3	VL-515-31...	108	107	- 1
LV-558-11...	98	94	- 4	VL-297-23...	98	109	+11
LV-569-22...	93	92	- 1	VL-297-19...	99	109	+10
LV-569-40...	93	95	+ 2	VL-297-5...	107	109	+ 2
LV-569-6...	96	92	- 4	VL-297-11...	121	109	-12
LV-569-23...	100	96	- 4				
LV-91-4....	102	102	0	Average	+0.5

To sum up, the fourth-generation families show either that selection for long pod had been effective in isolating plants homozygous for minor factors or that segregation of the residual minor factors was in most cases masked by the modifications.

SUMMARY

(1) A single genetic difference, *E*, is responsible for the main difference between short and long pods. This genetic difference segregates in normal Mendelian fashion.

(2) Factor *E* is completely quantitatively dominant, so that $E_2 = Ee$.

(3) This factor acts as a multiplier, with a multiplying value of about 1.51.

(4) Minor factors for pod length also act as multipliers, with a combined multiplying value (when double) of about 1.42.

(5) These minor factors apparently show zero dominance, in the sense that if $A_2 B_2 C_2 \dots$ are positive double factors with a combined multiplying value of x , the value of $AaBbCc \dots$ is \sqrt{x} .

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PLATE XL

Typical 5-seeded bean pods, showing the length of parents and crosses; *A*, One of the shortest second-generation pods; *B*, the Florida velvet-bean pod; *C*, the Lyon-bean pod; *D*, one of the longest second-generation pods.



A HONEYCOMB HEART-ROT OF OAKS CAUSED BY STEREUM SUBPILEATUM

By WILLIAM H. LONG,

Forest Pathologist, Investigations in Forest Pathology, Bureau of Plant Industry

INTRODUCTION

During investigations made in 1912, 1913, and 1914 on the pathological condition of the oaks (*Quercus* spp.) in the National Forests of Arkansas and in other sections of the United States, the writer found a large percentage of the trees, especially in some regions of Arkansas, attacked by various species of heart-rotting fungi. Among this number were several typical delignifying fungi: *Polyporus pilotae*; *P. berkeleyi*, and *P. frondosus*, which usually occur as butt-rots;¹ and *P. dryophilus*, which produces a widely distributed top-rot in oaks.² In addition to the rots produced by these four fungi, another type of rot was found in oaks which has certain characters not assignable to any fungus known to produce heart-rot in oaks. This undescribed rot is of the pocketed type (Pl. XLI, fig. 1) and is a typical delignifier of the heartwood. In the final stage of this rot the diseased wood resembles a piece of honeycomb (Pl. XLI, fig. 2). For this reason the writer calls it the "honeycomb heart-rot." The rot is very similar to that produced by *Stereum frustulosum* in dead standing or fallen oak timber, but is distinct from it.

The writer has repeatedly found this rot directly associated with the sporophores of *S. subpileatum*. The mycelium could easily be traced from the diseased wood to the subiculum of the sporophores. The only sporophores of this fungus found were in direct association with the typical honeycomb-rot.

DESCRIPTION OF THE HONEYCOMB HEART-ROT

The pocketed or honeycomb heart-rot caused by *S. subpileatum* was found by the writer to be directly associated with the sporophores of this fungus in the following nine species of oaks: *Quercus alba*,³ *Q. lyrata*, *Q. marilandica*, *Q. michauxii*, *Q. minor*, *Q. palustris*, *Q. texana*, *Q. velutina*, and *Q. virginiana*.

¹ Long, W. H. Three undescribed heart-rots of hardwood trees, especially of oak. In Jour. Agr. Research, v. 1, no. 2, p. 109-128, pl. 7-8. 1913.

² Hedgcock, G. G., and Long, W. H. Heart-rot of oaks and poplars caused by *Polyporus dryophilus*. In Jour. Agr. Research, v. 3, no. 1, p. 65-78, pl. 8-10. 1914.

³ The nomenclature for trees used in this paper is that of George B. Sudworth. (Check list of the forest trees of the United States, their names and ranges. U. S. Dept. Agr. Div. Forestry Bul. 17, 144 p. 1898.)

HONEYCOMB HEART-ROT IN WHITE OAK

MACROSCOPIC CHARACTERS

The first indication of this honeycomb heart-rot in white oak (*Q. alba*) is a slight discoloration of the heartwood, which assumes a water-soaked appearance. This "soak" may extend from 1 to 6 feet beyond the actually rotting region where delignification is occurring. When dry, the water-soaked heartwood becomes tawny in color.

Light-colored, isolated areas appear in the discolored wood. These areas, which are the beginnings of the pockets, usually originate in the region of the large vessels and often have a small medullary ray in their centers. The rot then spreads in all directions into the surrounding tissue, but moves more rapidly in the summer wood of the annual ring of the preceding year. This results in the bulk of the pocket lying in the summer wood of one year and the spring wood of the succeeding year.

The next stage of the rot is one of delignification in which very small irregular patches of delignified wood fibers appear in the light-colored areas. This delignification, which seems to begin in the wood fibers of the preceding year's growth of summer wood immediately adjacent to a large vessel, proceeds rapidly until white, oval to circular pockets appear (Pl. XLI, fig. 3). In radial section these lens-shaped pockets range from 5 to 15 mm. long by 1 to 5 mm. wide, with their main axes parallel to the grain of the wood. These pockets are at first filled with white cellulose (Pl. XLI, fig. 3 and 4), which later is gradually absorbed, leaving cavities lined with the remnants of the cellulose (Pl. XLI, fig. 5). Sometimes long lines of cellulose fibers extend longitudinally through several adjacent cavities, but, as a rule, the cellulose is limited to each individual pocket.

The attacked area increases in size until the pockets reach a large medullary ray on either side (Pl. XLI, fig. 6). These large rays seem to check the activity of the enzymes and therefore become the boundaries of the radial walls of the pockets. They are very evident even in the badly diseased heartwood (Pl. XLI, fig. 6). This is especially noticeable in tangential and cross-sectional views. Each pocket usually does not involve more than two annual rings of growth, unless the rings are very narrow, in which case several may be included. In cross section the rot shows as irregular to circular holes from 1 to 5 mm. in diameter lying between the large medullary rays.

All the cellulose finally disappears (Pl. XLI, fig. 2 and 7), leaving the pockets either (1) empty, (2) containing the shrunken white membranes of the included vessels, or (3) more or less filled with mycelium.

In the last stage of the rot the wood is very light and of a honeycomb-like structure (Pl. XLI, fig. 2 and 7). The pockets are longer than they are broad, and all of the wood has disappeared, except the thin walls surrounding the pockets, which remain distinct and usually involve the heartwood uniformly. The rotted wood is therefore in the shape of a cylinder.

There is a brownish discoloration of the heartwood on the outer edges of the affected area. This character is also common to several other heart-rotting fungi.

When a living tree having the rot caused by *S. subpileatum* is first split open, there is a very distinct odor of old honeycomb. In some white oaks the old pockets have blackish deposits on the walls which make this rot resemble even more strongly an old, blackened honeycomb.

MICROSCOPIC CHARACTERS

A microscopic examination of the diseased wood in the initial stage of a pocket shows small groups of partially delignified wood fibers scattered in the neighborhood of the large vessels. Delignification in these wood fibers begins with the inner layer or the tertiary lamella of each fiber and proceeds outward toward the primary or middle lamella. The middle lamella is then attacked and rapidly dissolved, thus freeing each cell from its neighbor.

The walls of the small medullary rays are more slowly delignified than the wood fibers, while the walls of the large vessels resist delignification much longer than either the wood fibers or small medullary rays. The tyloses in the large vessels are the last to be delignified. They contain many small, irregular holes, apparently made by the passage of fungus hyphæ through them. Delignification is not very pronounced in the cells of the radially placed rows of small vessels of the summer wood.

The pits of the vessels and the cells do not seem to be enlarged by the action of the fungus until the last stages are reached, if at all.

FUNGOUS MYCELIUM

In the earliest stages of the rot the enzymes seem to precede the fungous hyphæ, especially in the region of the wood fibers. In the larger vessels a few colorless very small hyphæ can be seen in the region adjacent to the area first delignified. As delignification advances, the threads in the vessels increase in number, and during the period of cellulose absorption the vessel from which the delignification started often becomes stuffed with small, intricately branched, colorless hyphæ.

In the center of the pockets are often seen small, white, threadlike bodies. On examination these prove to be (1) the remnants of the delignified walls of the vessels and especially of the tyloses, which often persist even after all of the walls of the vessels have been absorbed, and (2) fungous tissue, which is composed of large (10μ), longitudinal, hyaline, thin-walled hyphæ and many smaller hyphæ, all interwoven into a rodlike mass.

In many of the pockets where much of the cellulose has been absorbed, dense white fluffy masses of mycelium either nearly fill or in some instances only line the cavities. This mycelium is composed of small, branched, colorless, thick-walled hyphæ, some of which have granular or tuberculate walls. If the pockets border on checks or windshakes, the fluffy masses of mycelium are a reddish brown in place of white and often form a more

or less tough, brown mycelial web in the fissures of the wood. A similar mycelial growth often develops on specimens of freshly cut rotting wood from the exposed edges of the cellulose-filled cavities and may even overrun the surface of the rotting wood for several square inches.

This reddish growth seems to occur only when the actively growing hyphae are exposed to the air, since in the interior of the wood, where they are not thus exposed, the mycelium lining the original cavities caused by this fungus is white. The brownish mat of mycelium which forms in the fissures of the wood consists of dense interwoven masses of sparingly septate, fulvous hyphae. The clamp connections of these hyphae are not very pronounced, in marked contrast to those of *S. frustulosum*. These hyphae are from 2 to 3 μ thick, as a rule, but smaller ones are not uncommon with branches putting out at right angles to the main hypha. The outer walls of some of the hyphae are sparingly granular to almost tuberculate.

The very old pockets are often filled with a brownish floccose mass, which is composed of brown, tuberculate hyphae similar to those seen in the rot produced by *S. frustulosum*.

RESEMBLANCE OF THE ROT CAUSED BY STEREUM SUBPILEATUM TO CERTAIN OTHER ROTs

It is very difficult and often impossible to separate very similar types of rot from one another, unless the fruiting bodies of the causative organism are present in direct association with the rot.

There are four delignifying heart-rots which are very similar in certain stages of their development to each other and to portions of the description given by Von Schrenk and Spaulding¹ of a piped-rot of oak and chestnut. In the light of recent investigations these four rots are now known to be caused by the following fungi: (1) *Polyporus dryophilus*, which causes a very common heart-rot in the upper portion of the trunks of oaks in the United States and is found occasionally in poplars; (2) *P. pilotae*, which attacks the heartwood of oaks and chestnuts; (3) *Stereum subpileatum*, which causes a pocketed-rot of oaks; and (4) *Hymenochaete rubiginosa*, which causes a pocketed-rot in chestnut and oak. The writer has specimens of the last-named fungus, collected during the past three years in several States and associated with a delignifying pocketed heart-rot in living chestnut. On account of the meagerness of the sporophore material, the writer was uncertain whether *H. rubiginosa* was really the cause of the rot with which it was associated or was only a secondary fungus on already diseased chestnut timber. Brown in a recent article² describes a pocketed-rot in dead chestnut and oak timber with which the sporophores of *H. rubiginosa* are constantly associated. However, he did not find it as a heart-rot in living trees.

¹ Schrenk, Hermann von, and Spaulding, Perley. Diseases of deciduous forest trees. U. S. Dept. Agr. Bur. Plant Indus. Bul. 149, 85 p., 11 fig., 10 pl. 1909.

² Brown, H. P. A timber rot accompanying *Hymenochaete rubiginosa* (Schrad.) Lév. In *Mycologia*, v. 7, no. 1, p. 1-20, pl. 149-151. 1915.

COMPARISON OF ROTS OF STEREOUM SUBPILEATUM AND POLYPORUS PILOTAE

In the writer's investigation in the Ozarks no attempt was made in the field to separate the rot caused by *P. pilotae* from that caused by *S. subpileatum*, since both in their early stages produce small delignified areas in the diseased heartwood of living trees. It was therefore difficult to determine which fungus produced the rot unless the sporophores were present. Attention was called to this resemblance in a previous article by the writer.¹ However, the final stage of the rot produced by *P. pilotae* is quite distinct from that of *S. subpileatum*. The rot caused by *P. pilotae* usually moves upward in the infected wood, along certain well-defined zones consisting of several annual rings of growth of the wood. These zones are usually separated by zones of apparently sound tissue—that is, the rot moves upward or longitudinally in the tree more rapidly than it does radially. The rot caused by *S. subpileatum* does not seem to form definite zones of infected wood separated by sound zones, at least in the white oak, but seems to move as rapidly radially as longitudinally in the attacked heartwood, thus forming a uniform cylinder of rotted wood in the heartwood of the trees. If this character should prove constant, one could use it in field work for differentiating this rot from the earlier stages of the rot of *P. pilotae*. However, in well-advanced stages of rot, the presence of typical lens-shaped to cylindrical pockets occupying practically all of the infected heartwood is fairly indicative that the rot in question is caused by *S. subpileatum*.

ENTRANCE OF THE FUNGUS INTO THE HOST

The fungus *S. subpileatum*, so far as the writer knows, enters the wood of the hosts only through wounds which expose the heartwood. The most common point of entrance is through wounds, usually fire scars, in the butt of the trees, although it also frequently enters through branch stubs. The writer found this rot several times in the tops of living white-oak and black-oak trees in the Ozark National Forest, Arkansas. In every case the fungus had undoubtedly entered through a branch stub. It produces the same type of rot (Pl. XLI, fig. 4 and 7) in the tops as it does in the butts, even to the peculiar honeycomb-like odor.

No instances were found where this rot had entered a living tree through the dead sapwood of a wound, nor where it had entered a dead tree or log through the sapwood. It is very probable, however, that the fungus does attack dead timber in this manner, since many examples were found where the fungus had grown from the heartwood into the dead sapwood of felled trees.

¹Long, W. H. Three undescribed heart-rots of hardwood trees, especially of oak. *In Jour. Agr. Research*, v. 1, no. 2, p. 109-128, pl. 7-8. 1913.

SPOROPHORE OF STEREOUM SUBPILEATUM

The sporophores of *S. subpileatum* have been found by the writer only on dead trees or on dead areas on living trees. They usually occur on the fallen trees which had this rot while living. *S. subpileatum* apparently does not attack the living sapwood and therefore has no chance to fruit unless the diseased heartwood is exposed by the death of the tree or by the breaking off of the trunk or of a branch. When an oak whose heartwood is attacked by this fungus is felled, the fungus continues to grow in the heartwood of the felled tree (Pl. XLI, fig. 8) and also grows outward into the sapwood. When the actively growing mycelium reaches the surface of the sapwood, the thin shelving sporophores (Pl. XLI, fig. 9) are formed in the cracks between the bark, or if the bark has been burned off or has fallen off, large numbers of sporophores, often conchate in shape (Pl. XLI, fig. 10), are formed over the entire surface of the fallen tree. These sporophores usually form in long, continuous parallel lines. The individual sporophores range from 0.25 to 2 inches in width, depending on their age.

Living trees with this rot when felled usually lie for two or more years before any sporophores are formed. After sporophore formation once commences, the sporophores usually continue to grow for many years; therefore a tree or log culled for this rot in a lumbering operation, if not destroyed, will after one or two years be a menace for years to the future health of the forest.

DESCRIPTION OF THE SPOROPHORE OF STEREOUM SUBPILEATUM

Pileus rather thick, medium-sized, coriaceous, firm, drying rigid and hard, sessile, dimidiate, conchate, subimbricate, often laterally connate, usually effuso-reflexed, decurrent onto the wood for 0.5 to 2 cm., 1 mm. thick by 0.5 to 6 cm. wide (measured from front to rear of sporophore) and 2 to 12 cm. or more broad, perennial, attached to substratum by a thin subiculum of densely woven Mars yellow¹ hyphæ; surface finely tomentose at first, becoming glabrate with age, multizonate, older zones drab gray, finally becoming very indistinct and nearly glabrous, often radiately furrowed, marked with several concentric furrows of variable width and depth; margin thin, undulate, often incurved, strongly tomentose, tomentum from light buff to Mars yellow; hymenium inferior, sometimes stratose, changing color when injured and moistened, often concave, even, light buff; basidia simple with four sterigmata; spores colorless, even, broadly oval, flattened on one side, 4 to 5 by 3 μ ; cystidia incrustated, colorless, becoming brownish where buried in older layers of the hymenium, cylindrical, 25 to 40 by 6 to 8 μ , not present in the intermediate or tramal layer.

¹ Ridgway, Robert. Color Standards and Color Nomenclature. 43 p., 53 col. pl. Washington, D. C., 1912.

DISTRIBUTION OF STEREOUM SUBPILEATUM

The rot caused by *S. subpileatum* is rather widely distributed in certain sections of the United States, having been found in eight States: Arkansas, Kentucky, Florida, Louisiana, Mississippi, Missouri, Ohio, and Virginia. Authentic specimens of the fungus have also been examined from Mexico. The sporophores of the fungus are frequent and the rot caused by it is common in Arkansas, Mississippi, and Louisiana.

DISTRIBUTION IN AMERICA

S. subpileatum has been reported from and collected in the various States of this country as follows:

ARKANSAS:

On *Quercus alba*.—Casteel, LONG, in August and December, 1912 (F. P. 12136,¹ 12178, 12194, 12629, 12729, 18619, 19026); Arkansas National Forest, LONG, in September, 1913 (F. P. 12703, 19016).

On *Quercus nigra*.—Arkansas City, LONG, in November, 1913 (F. P. 19065).

On *Quercus palustris*.—Arkansas City, LONG, in November, 1913 (F. P. 18405).

On *Quercus phellos*.—Arkansas City, LONG, in November, 1913 (F. P. 19064).

On *Quercus rubra*.—Lake Village, LONG, in November, 1913 (F. P. 19071).

On *Quercus texana*.—Arkansas National Forest, LONG, in September, 1913 (F. P. 18502, 18715).

On *Quercus velutina*.—Arkansas National Forest, LONG, in September, 1913 (F. P. 12567, 12724); White Rock, LONG, in September, 1912 (F. P. 12242).

FLORIDA:

On *Liquidambar styraciflua*.—(?) G. C. FISHER (No. 07643, Herb. Lloyd).

On *Quercus* sp.—C. G. LLOYD, in February, 1899 (No. 4846, Herb. Lloyd); Lake City, ROLFS and FAWCETT, in March, 1906 (Herb. Lloyd).

On *Quercus virginiana*.—New Smyrna, LONG, in March, 1914.

On *Quercus* sp. (?).—Gainesville, H. S. FAWCETT (No. 08090, Herb. Lloyd).

On wood.—G. C. FISHER (No. 07849, Herb. Lloyd).

KENTUCKY:

On *Quercus* sp. (?).—Mammoth Cave, C. G. LLOYD, in July, 1897 (No. 2798, Herb. Lloyd).

LOUISIANA:

On prostrate logs, St. Martinsville, LANGLOIS, in April, 1897 (No. 2428, Herb. Lloyd No. 5).

On *Quercus lyrata*.—Lutcher, LONG, in November, 1913 (F. P. 19067, 19091).

MISSISSIPPI:

On *Quercus phellos*.—Stoneville, LONG, in November, 1913 (F. P. 18722).

MISSOURI:

On *Quercus palustris* (?).—Steelsville, SPAULDING No. 44 (F. P. 12955).

OHIO:

On wood.—Cincinnati, A. P. MORGAN (Herb. Lloyd).

VIRGINIA:

On *Quercus alba*.—Great Falls, LONG, in 1914.

On *Quercus coccinea*.—Veitch, LONG, in 1913 (F. P. 12571).

On *Quercus prinus*.—Arlington, LONG, in 1914.

On *Quercus velutina*.—Park Lane, LONG, in 1914.

¹ "F. P." = Forest-Pathology Investigations number.

MEXICO:

On *Quercus* (?).—Jalapa, CHARLES L. SMITH, No. 146 Central American Fungi, in 1894 (No. 4709, Herb. Lloyd).

From the foregoing data it will be noted that the following trees are attacked by the disease caused by *S. subpileatum*: *Q. alba*, *Q. coccinea*, *Q. lyrata*, *Q. marilandica*, *Q. michauxii*, *Q. minor*, *Q. palustris*, *Q. phellos*, *Q. prinus*, *Q. rubra*, *Q. texana*, *Q. velutina*, *Q. virginiana*, *Quercus* spp., and *Liquidambar styraciflua* (?).

CONTROL OF THE HONEYCOMB HEART-ROT CAUSED BY *STEREUM SUBPILEATUM*

The honeycomb heart-rot caused by *S. subpileatum* is one of several important heart-rots of oaks in the United States. Suggestions made for its control will apply more or less to all of these. The fact that apparently oaks of all ages are susceptible to this rot, provided they are old enough to have formed heartwood, must be taken into consideration when discussing methods of control. The only practicable method of control known which can be applied to the forest as a whole is to prevent, so far as possible, the infection of the trees. This can be done (1) by eliminating, so far as possible, all forest fires, since they produce wounds on the butts of the trees through which the fungus enters; (2) by preventing the formation of the fruiting bodies (sporophores) of the fungus which produce the spores. These spores are the direct agents for infecting the trees through dead branches and fire scars.

The only method at present known by which the development of the sporophores of this fungus can be prevented is the destruction of all diseased timber which contains this rot. In lumbering tracts of oak all unsound or diseased trees should be cut, the parts that can be used removed, and the cull logs and dead trees burned, since this fungus fruits most abundantly on old logs and on dead fallen timber. Many trees under the present methods of lumbering are left standing because they have heart-rot in the butt. If cut down, these trees would usually be found to contain enough lumber to pay for the cost of operation. Such a procedure will lead to a better and closer utilization of our gradually decreasing supply of oak and insure a healthier future forest.

Special emphasis should be placed on the fact that the rot produced by *S. subpileatum* can continue to grow in a tree after it is felled, and that every cull butt, log, or tree left on the ground in a lumbering operation will later bear an enormous number of sporophores of this fungus which will discharge annually millions of spores for many years. In the interest of the health of the future forest, it is therefore of the utmost importance that all of these cull logs and trees be destroyed.

PLATE XLI

Fig. 1.—*Quercus alba*: A radial view of the honeycomb heart-rot produced by *Stercum subpileatum*, showing various stages of the rot; from Arkansas.

Fig. 2.—*Quercus alba*: A radial view of the last (honeycomb) stage of the rot; from Arkansas.

Fig. 3.—*Quercus alba*: A tangential view of honeycomb-rot, showing early stage of delignification; from Arkansas.

Fig. 4.—*Quercus velutina*: A radial view of honeycomb heart-rot as it occurs in tops of trees, showing pockets filled with strands of cellulose; from Arkansas.

Fig. 5.—*Quercus alba*: A radial view of the honeycomb-rot, showing pockets lined with cellulose; from Arkansas.

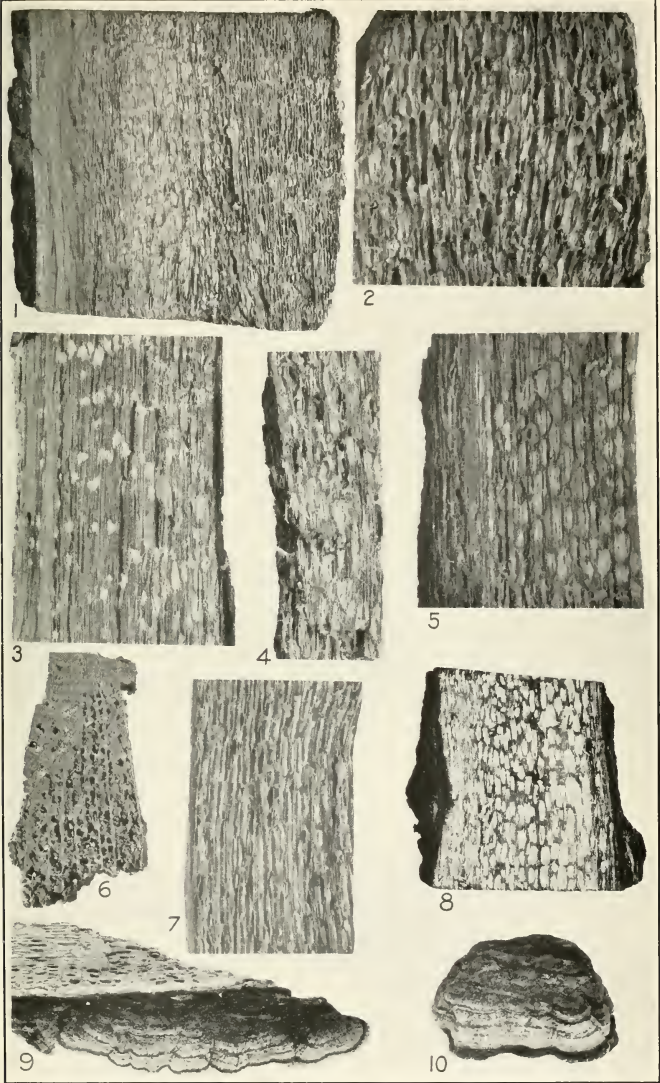
Fig. 6.—*Quercus alba*: A cross-sectional view of the honeycomb heart-rot, showing pockets limited by large medullary rays; from Arkansas.

Fig. 7.—*Quercus alba*: Radial view of honeycomb heart-rot in branch, showing last stage of rot; from Arkansas.

Fig. 8.—*Quercus lyrata*: Radial view of honeycomb heart-rot in old log associated directly with the sporophores of *S. subpileatum*; from Louisiana.

Fig. 9.—*Quercus texana*: Sporophore of *S. subpileatum*; from Arkansas.

Fig. 10.—*Quercus palustris*: Sporophore of *S. subpileatum*, conchate form; from Arkansas.



MEASUREMENT OF THE WINTER CYCLE IN THE EGG PRODUCTION OF DOMESTIC FOWL¹

By RAYMOND PEARL,

Biologist, Maine Agricultural Experiment Station

In a series of papers the writer and his associates (2, 6, 9)² have shown that there are to be distinguished definite cycles in the egg-laying activities of the fowl. The two most striking and definite of these cycles we have called, respectively, the "winter" and the "spring" cycles, these terms being used because of the seasonal incidence of these periods of laying activity. In the writer's studies on the inheritance of fecundity (4, 5, 7, 8) in the fowl he has used as an index of the innate fecundity of a bird its pullet-year "winter production," defined as the number of eggs produced before March 1 of the bird's pullet year—i. e., the first March 1 following the individual's birth. The reasons why this measure of productivity rather than some other was chosen for the work have been fully set forth in earlier papers and need not again be gone into here. It may suffice to say that, by all the tests which it has so far been possible to apply, this index of fecundity has proved very satisfactory in practice. The results which one obtains with it are duplicated in every essential particular if one uses the longer period of one year, but genetic differences in fecundity are more strongly emphasized in the shorter period, with a corresponding gain in the precision and certainty of the Mendelian analysis.

It has never been contended, however, in any of the writer's work that winter production, as above defined, was anything more than an index or indicator of innate fecundity. It is logically obvious that the only perfect measure of total fecundity would be some direct function of total fecundity. All that the writer's work has shown regarding the point here under discussion is that winter production is a good indicator, all things considered, of a fowl's innate fecundity capacity. It is not a perfect indicator, but that it is a good one is confirmed not only by the experience of this laboratory but also by that of other workers (1, 3, 10).

In the course of the writer's investigations regarding this character, studies have been made of various other fecundity indicators besides winter production. The thought occurs to one that possibly under other environmental conditions than those prevailing in Maine winter produc-

¹ Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 89.

² Reference is made by number to "Literature cited," pp. 436-437.

tion might prove a less valuable and reliable indicator. This may possibly be so, though up to the present time no definite evidence on the point has appeared. Another point which occurs to one is that possibly a better measure of the winter cycle of productivity (this being the biological entity we attempt to measure by the record of production to March 1) might be obtained by using the egg production of a bird up to the time when it has attained a definite age. Fowls are hatched at different dates, while March 1 is a fixed point in time. Birds hatched at different times will be of different ages at March 1 of their pullet year. Will the egg production prior to the attainment of a definite age by a bird give a better measure of her winter cycle than the production prior to a fixed date without regard to age, except so far as this is involved in having the birds all hatched within a certain limited season? It is the purpose of this paper to present some data on this question.

Specifically the material here presented has to do with the suggestion that the egg production up to 300 days of age of the bird gives a better measure of the winter cycle than does the production to March 1, since an age of 300 days will include the winter cycle, and will also allow for differences due to variation in date of hatching. Biometrically we can readily test this question in two ways: On the one hand, we can determine the correlation between the winter production as defined by the writer (to March 1) and the production to 300 days of age, on the other hand. If this correlation is low, it will mean that one of the measures is probably sensibly better than the other. If, on the other hand, the correlation is very high, differing but little from perfect correlation, it will indicate, so far as it goes, that there is little to choose between the two measures. In the second place, we may examine the variabilities biometrically. On theoretical grounds that measure of a character is best, other things being equal, which exhibits the smallest relative variability.

Evidence along these lines derived from extensive trap-nesting experiments is presented in the following tables. The data cover three consecutive years. Two correlation tables are presented for each year: One including the total flock of that year regardless of breed distinctions, the other including only pure Barred Plymouth Rocks. The total flocks were made up of various crossbred birds used in Mendelian experiments, in addition to the pure Barred Plymouth Rocks. All birds included in the tables are pullets—i. e., they were hatched in the spring of the year indicated in the caption of the table. The computations were made by Mr. John Rice Miner, staff computer of the Biological Laboratory. See Tables I to VI.

TABLE I.—Correlation between (a) egg production to March 1, and (b) egg production to 300 days of age, for pure Barred Plymouth Rocks hatched in 1911

EGG PRODUCTION TO 300 DAYS OF AGE.																			Total.
0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84			
8																		8	
1	1																	3	
10	1	2	1															5	
15	1	1	2															5	
20			3	6	5	1												15	
25				5	4	5												19	
30			1	1	7	6	3	1										14	
35				2	1	6	5											16	
40						7	5	2										18	
45						4	4	1	1									23	
50							3	12	3									22	
55								9	4	3								10	
60								5	4	6	1							12	
65								3	3	3	1							12	
70										3	2	4						6	
75											2	3	1					2	
80												2						4	
85											3							2	
90												1							
95															1				
99																			
100-104																	3	3	
Total	11	4	7	17	17	29	21	22	16	15	13	10	10	1	2	1	3	199	

TABLE II.—Correlation between (a) egg production to March 1 and (b) egg production to 300 days of age, for total flock hatched in 1911

		EGG PRODUCTION TO 300 DAYS OF AGE.																	Total.
		0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	
EGG PRODUCTION TO MARCH 1.	0-4	37	7	6															50
	5-9	13	8	6	5	1													34
	10-14	6	8	6	2	2													31
	15-19	1	5	8	6	6	3												32
	20-24			8	17	11	2	1											39
	25-29			2	11	11	11	2											38
	30-34				1	17	13	6	1										39
	35-39					2	10	7	3	2									26
	40-44					1	9	9	5	5	1				1				32
	45-49						4	4	15	7	1								35
	50-54							4	19	4	6	1							38
	55-59								4	8	1	1							15
	60-64								2	1	3	2	1		1				18
	65-69										5	1	1						14
70-74											3	4	4	4	2			2	
75-79												2	4			1		11	
80-84													2					5	
85-89												1	3			1		2	
90-94														1					
95-99																			
100-104																		3	
Total		57	28	40	49	51	55	33	31	39	21	24	15	13	2	2	1	3	464

TABLE III.—Correlation between (a) egg production to March 1 and (b) egg production to 300 days of age, for pure Barred Plymouth Rocks hatched in 1912

		EGG PRODUCTION TO 300 DAYS OF AGE.																Total.	
		0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79		80-84
EGG PRODUCTION TO MARCH 1.	0-4	6																	6
	5-9	3	3																6
	10-14	1	1	1															3
	15-19		2	2	1														5
	20-24	4		1	2														7
	25-29		1	1	2	3	1												8
	30-34		1		1	4	2	2											10
	35-39			2	2	3	3	8	5	1		1							22
	40-44				2	5	2	3	6	6	1								19
	45-49				2	1	5	3	6	5	5	3							19
	50-54					1	1	1	3	6	3	3							22
	55-59								3	2	2	2	2						16
	60-64								2	2	2	1	3	2					7
	65-69									2	2	1	1	2					17
	70-74										1	2	1	1					6
	75-79										2	4	1	2	1				9
80-84												1	2	2				5	
85-89													4				2	6	
90-94																1	1	3	
95-99																1	1	2	
100-104																			
105-109																	1	1	
Total.....		14	8	7	10	17	19	19	20	16	22	14	9	11	4	3	4	2	199

TABLE IV.—Correlation between (a) egg production to March 1 and (b) egg production to 300 days of age, for total flock hatched in 1912

		EGG PRODUCTION TO 300 DAYS OF AGE.																	Total.
		0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	
EGG PRODUCTION TO MARCH 1.	0-4	42	3			1													46
	5-9	9	10	3															23
	10-14	9	3	8	2	2	1												25
	15-19	1	5	6	9	1	1												22
	20-24			3	11	7	6	1											36
	25-29		2	3	5	8	12	3	2	1									36
	30-34		1		5	7	9	4	3		1								40
	35-39			2	3	7	16	13	9	1		2							53
	40-44				2	7	8	6	11	6									42
	45-49					7	7	8	4	6	2	2							31
	50-54					1	2	6	4	8	9								33
	55-59							11	2	3	3	6	3		1				27
	60-64								3	4	2	2	3	1		1			15
	65-69									2	10	3	2	3	1				20
	70-74									1	2	1	1	2	2		1		8
	75-79										4	1	1	2	1				9
	80-84											1	1	2	3				6
85-89														4			2	6	
90-94																1	1	3	
95-99																1		2	
100-104																			
105-109																	1	1	
Total		68	24	25	39	53	61	42	47	31	30	25	12	13	5	3	4	2	484

TABLE V.—Correlation between (a) egg production to March 1 and (b) egg production to 300 days of age, for pure Barred Plymouth Rocks hatched in 1913

EGG PRODUCTION TO 300 DAYS OF AGE.																								
	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-99	100-104	105-110	Total.	
0-4	10																						11	
5-9		2																					3	
10-14			1																				1	
15-19				1																			5	
20-24				3	2																		9	
25-29				1	3	3																	7	
30-34				2	3	1	1	1															14	
35-39				1	3	2	3	3	3		2												7	
40-44				3	3	2	3	3	1	1													18	
45-49				1	2	2	3	3	5	2													18	
50-54							2	2	1	4	8												17	
55-59								1	1	5	6	4											14	
60-64										1	3	5	4										12	
65-69											3	5	4	4									12	
70-74												5	4	3	3								16	
75-79													4	3	6	3							14	
80-84														4	2	3	1						11	
85-89															1	2	3	3	1				12	
90-94															2	4	3	3	1				6	
95-99															3	1	1	1	1				1	
100-104																1							1	
105-109																	2	1	3				6	
110-114																							1	
115-119																							1	
120-124																							1	
125-129																							1	
Total...	10	2	3	4	8	8	9	16	10	14	19	21	14	14	15	14	13	9	7	3	2	2	217	

TABLE VI.—Correlation between (a) egg production to March 1 and (b) egg production to 300 days of age, for total flock hatched in 1913

	EGG PRODUCTION TO 300 DAYS OF AGE.																							Total.
	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-99	100-104	105-109		
0-4...	37	3	2	1	1																		43	
5-9...	11	5	3	1	1																		18	
10-14...	5	3	3	1	4	2																	18	
15-19...	2	4	4	3	3	2																	20	
20-24...	...	3	4	6	3	7																	20	
25-29...	...	2	3	3	7	3																	28	
30-34...	...			3	7	5																	18	
35-39...	...			1	7	5																	29	
40-44...	...			1	7	5																	18	
45-49...	...			1	7	5																	29	
50-54...	...			1	7	5																	18	
55-59...	...			1	7	5																	29	
60-64...	...			1	7	5																	18	
65-69...	...			1	7	5																	29	
70-74...	...			1	7	5																	29	
75-79...	...			1	7	5																	29	
80-84...	...			1	7	5																	29	
85-89...	...			1	7	5																	29	
90-94...	...			1	7	5																	29	
95-99...	...			1	7	5																	29	
100-104...	...			1	7	5																	29	
105-109...	...			1	7	5																	29	
110-114...	...			1	7	5																	29	
115-119...	...			1	7	5																	29	
120-124...	...			1	7	5																	29	
125-129...	...			1	7	5																	29	
Total...	55	20	23	18	30	26	26	25	18	31	34	33	28	24	23	20	14	14	9	3	2	2	478	

It is evident from mere inspection of Tables I to VI that the correlation between these two variables is very high and that the regression is linear. Calculating the coefficients of correlation by the usual Bravais formula, $r = \frac{S(xy)}{N\sigma_1\sigma_2}$, with a probable error of r given by the expression $PE_r = \pm .67449 \frac{1-r^2}{\sqrt{n}}$, we have the results set forth in Table VII.

TABLE VII.—Coefficients of correlation between (a) egg production to March 1 and (b) egg production to 300 days of age

Year.	Flock.	Coefficient of correlation.
1911....	Barred Plymouth Rock.....	0.955 ± 0.004
1911....	Total.....	.939 ± .004
1912....	Barred Plymouth Rock.....	.923 ± .007
1912....	Total.....	.915 ± .005
1913....	Barred Plymouth Rock.....	.949 ± .005
1913....	Total.....	.921 ± .005

These coefficients are clearly of a high order of magnitude. They fall in the same class, for example, as coefficients measuring the correlation between homologous organs on the two sides of bilaterally symmetrical organisms. These values in the present case lead unequivocally to the conclusion that with the flocks of birds here considered there certainly is no definite or marked superiority of either of these measures of the winter cycle of productivity over the other. These high correlations indicate that the two measures can be employed interchangeably so far as practical statistical work is concerned. This does not mean that the records to March 1 and to 300 days will be identical for a particular hen. What the high correlations do mean is that if an individual, A, has a higher record to March 1 than another individual, B, the probability is so high as to amount nearly to certainty that A will also have a record to 300 days which will be higher than the corresponding record of individual B and by an amount in proportion to the difference exhibited by the records to March 1.

It will be noted that the correlation for the total flock is lower than that for the Barred Plymouth Rock flock in every case. No biological significance appears to attach to these differences, which are small in amount.

The three years here dealt with are entirely typical, and an examination of our data indicates clearly that precisely the same result would be reached if we used the material from other years of the trap-nest records of the Maine Station. There was felt to be no point in piling up further correlation coefficients, all showing the same thing. The figures given above are quite sufficient to show that there is no warrant what-

ever for the assertion that the record to 300 days of age is a better measure of the winter-cycle production than is the record to March 1, so far as concerns the flocks which have been used in the writer's investigations of fecundity. Of course, it might possibly be that if one did the bulk of his hatching very late in the season, so that the pullets were not properly matured in the fall, then the 300-day record might be more reliable than the March 1 record. Tables I to VII demonstrate, however, that there is no distinct or marked superiority of one of these measures over the other when the flocks are bred and managed as those of the Maine Station have been during the last eight years.

We may turn now to an examination of the variation constants for the two measures. These are shown in Table VIII.

TABLE VIII.—*Variation constants for (a) egg production to March 1, and (b) egg production to 300 days of age*

EGGS LAID BEFORE MARCH 1

Year.	Flock.	Mean.	Standard deviation.	Coefficient of variation.
1911....	Barred Plymouth Rock.....	43. 13±0. 97	20. 26±0. 69	46. 98±1. 91
1911....	Total.....	32. 45±. 67	21. 53±. 48	67. 26±2. 06
1912....	Barred Plymouth Rock.....	48. 41±1. 04	21. 83±. 74	45. 09±1. 81
1912....	Total.....	36. 24±. 68	22. 09±. 48	60. 96±1. 75
1913....	Barred Plymouth Rock.....	59. 37±1. 21	26. 39±. 85	44. 44±1. 70
1913....	Total.....	47. 68±. 89	28. 85±. 63	60. 51±1. 74

EGGS LAID BEFORE 300 DAYS OF AGE

1911....	Barred Plymouth Rock.....	34. 39±0. 83	17. 40±0. 59	50. 60±2. 10
1911....	Total.....	27. 09±. 56	17. 76±. 39	65. 57±1. 98
1912....	Barred Plymouth Rock.....	35. 97±. 91	19. 11±. 65	53. 12±2. 25
1912....	Total.....	28. 28±. 56	18. 15±. 39	64. 16±1. 39
1913....	Barred Plymouth Rock.....	54. 56±1. 12	24. 38±. 79	44. 68±1. 71
1913....	Total.....	42. 38±. 83	26. 92±. 59	63. 53±1. 86

From Table VIII it is apparent that, in the first place, the mean production for the 300-days-of-age group is uniformly below the mean production to March 1. Since the latter period can hardly be regarded as essentially overestimating the winter cycle, as judged on the basis of curves of the distribution of production through the year (9), clearly the 300-day grouping must somewhat underestimate in the case of flocks with a mean hatching date falling in the month of April. All the flocks which have been used in the study of fecundity at the Maine Station and on which all of our conclusions have been based have their mean date of hatching in the month of April. It is therefore plain that the 300-day measure can not in this respect be considered so good a measure of the winter cycle under the conditions prevailing in the writer's investigations as the March 1 measure.

It will be noted that the Barred Plymouth Rock means are higher throughout than are the total flock means. This merely signifies that in the total flocks are included many crossbred birds carrying low fecundity genes.

Turning to the coefficients of variation, which measure the relative variability, it is seen that in every case but one (total flock, 1911) the coefficient is lower for the March 1 than it is for the 300-day measure. The differences are, in the single instances taken by themselves, usually not statistically significant, having regard to the probable errors; but the general trend is unmistakably in the direction of a lower relative variability of the production to March 1, indicating again that this is a somewhat better measure of the winter cycle than the production to 300 days of age under the conditions prevailing in this work.

SUMMARY

In this paper quantitative evidence is presented which shows, with flocks of poultry having average hatching dates falling somewhere within the month of April, that—

(1) The correlation between the egg production to March 1 of the pullet year as one variable and the egg production up to the time when the individual is 300 days of age as the second variable is extremely high.

(2) The mean production to March 1 is, in general, higher than the mean production to 300 days of age.

(3) The production to March 1 is a relatively less variable measure (as indicated by the coefficient of variation) than the production to 300 days of age.

(4) The conclusion that the 300-day production would be a better measure of the winter cycle of fecundity than the production to March 1 is not warranted by the facts. Whatever superiority there is of one of these measures over the other is entirely in favor of the production to March 1. We may therefore conclude that the use, in the writer's investigations on fecundity, of the record of egg production to March 1 of the pullet year as a measure of the winter cycle of production is fully justified by a critical examination of the facts. The justification for the employment of the winter cycle of production as an index of innate fecundity capacity or ability is a distinct and separate problem which has been discussed at length in earlier papers.

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INFLUENCE OF GROWTH OF COWPEAS UPON SOME PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES OF SOIL

By C. A. LECLAIR,¹

Assistant Professor of Soils, University of Missouri

INTRODUCTION

In the past 25 years much experimental work has been done with cowpeas (*Vigna sinensis*) in relation to cultural methods, fertilization, and variety tests, but practically nothing has been written with regard to the direct effect of the plant upon the soil. Some have expressed the belief that cowpeas are capable of producing a loosening effect upon the soil, but no authentic experimental data are available.

HISTORICAL SUMMARY

An exhaustive study of research literature revealed that previous work along the particular line referred to has been exceedingly limited. The data at hand bear only indirectly upon the work of this experiment, but are worthy of consideration.

With regard to the effect of shading on soil, Bühler² reports having carried on an experiment on four broad plots of ground. One was exposed to sun and wind; the others were shaded by horizontal wooden trellises placed around each plot 40 cm. above the ground and so arranged as to cut off one-fourth, one-half, and three-fourths of the sunlight from respective screened plots.

Data at the end of the experiment showed that at midday the shaded plots had a lower temperature than the open plot by from 2 to 10 degrees centigrade. However, the cooling by night under the shaded plot was very slight, being less than 2 degrees centigrade, which explains the effectiveness of a windbreak in preventing injury by frost. In rainy weather the variation of temperature either by day or by night was much smaller.

The relative evaporation from plots throughout the test was as follows:

Treatment.	Percentage of evaporation.
No shade	100
One-fourth shade	84
One-half shade	71
Three-fourths shade	62

¹ The writer desires to acknowledge his gratitude to Prof. M. F. Miller, of the Missouri Experiment Station, under whose direction these experiments were carried out.

² Bühler, A. Influence des treillis abris sur la température du sol et sur l'évaporation. *In* Ciel et Terre, ann. 17, no. 1, p. 21-22. 1896.

Wollny¹ reports that the shade of crops on land has little or no tendency to increase the looseness of a soil, but his data show that a crop, either cereal or legume, partially prevents the land from becoming compact. He has proved that not alone is this effect due to the elimination of the effects of beating rains and sunlight thereafter but to a greatly increased bacterial activity on cropped land. The bacteria thrive better in the moderate shade afforded by the plants, produce more humus, and thus improve the soil structure. The author gives definite experimental data to substantiate his conclusions.

Stewart,² in experiments with the effect of shading on soil conditions, where tobacco under tents and in the open was grown for comparison, reports the following conclusions from his investigation. The soil under the tent remains more moist than the uncovered soil, a condition which is especially important during the dry growing period. For this reason the shaded soil is always closer to the optimum water content. Because the soil is not subject to the packing due to alternate wetting and drying, it remains in better physical condition.

PLAN OF THE WORK

The soil of the Missouri Experiment Station field, upon which this experiment was performed, analyzed as a silt loam. The surface soil to a depth of 8 inches is a grayish to brownish silt loam; from 8 to 21 inches it grades heavier and is dark red in color, and from 24 to 48 inches it becomes more granular, contains some sand, and is of a light yellowish tinge. The mechanical analysis is as follows: Fine gravel, 0.26; coarse sand, 0.37; medium sand, 10.77; fine sand, 0.77; very fine sand, 29.37; silt, 49.55; clay, 8.88; total, 99.97; volatile matter, 4.91.

This soil might be termed the Shelby silt loam, according to the classification of the United States Bureau of Soils

Work was actively begun on the preliminary part of this investigation in 1911. The number of samples of soil to be taken from the plots for analyses in order to eliminate the errors of sampling was determined by careful trials. Again, it was necessary to experiment with a mechanical device for measuring the compactness of the soil under different treatments.

A systematic plan for sampling the plots and for making tests for compactness at periodic times was arranged so as to avoid all chance of duplication of trials on the same piece of ground.

Experimental work was necessary upon a shade device that would permit rain to pass through without much hindrance and would shut out effectively the direct rays of the sun, thus providing the desired shade effect.

¹ Wollny, Ewald. *Der Einfluss der Pflanzendecke und Beschattung* . . . p. 165. Berlin, 1877.

² Stewart, J. B. Effects of shading on soil conditions. U. S. Dept. Agr. Bur. Soils Bul. 39, 19 p., 7 fig., 4 pl. 1907.

No crop was planted on plot D, which was unplowed and kept clean (Pl. XLII, fig. 1). Plot E was also unplowed, but was planted to cowpeas (Pl. XLII, fig. 2). Plot F was plowed and planted to cowpeas (Pl. XLII, fig. 1). No crop was planted on plot G, which was plowed, artificially shaded, and kept clean (Pl. XLII, fig. 2). Plot H was also plowed and kept clean, but was without shade or cowpeas (Pl. XLII, fig. 2).

The plots were laid out on May 31, 1912. Plots F, G, and H were carefully spaded at this time. Plots D and E were scraped with a hoe to remove trash and weeds, but no further treatment was given. A week later, on June 11, plots E and F were drilled to Black cowpeas with an ordinary wheat drill, dropping the cowpeas in rows 8 inches apart at the rate of $1\frac{1}{2}$ bushels per acre. The drill was operated by pulling it at the end of a long rope so that the horses were not permitted

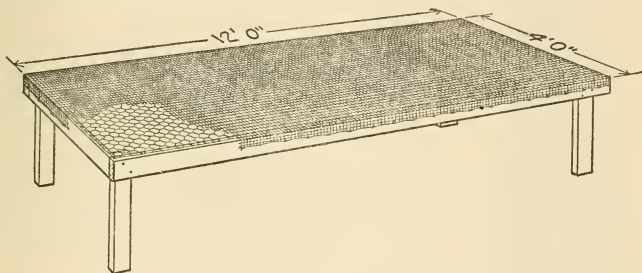


FIG. 1.—Soil-shading device, showing construction.

to walk over the plots. On June 9, after planting, all plots were gently scraped with a hoe to give them an equal start.

The main point at issue was a study of the soil compactness and nitrate content of plots in relation to the various treatments at the beginning and end of the growing season. An artificial shade was erected on plot G at a time when the cowpeas on plots E and F were matting over the soil. The shade device was a frame made of 2- by 4-inch lumber supported on legs made of the same material (fig. 1). Over this some galvanized screen was tightly stretched to serve as a support for a thin piece of black cheesecloth, which was found to be efficient in shading the soil from the direct rays of the sun and still only slightly impeding the rain.

Tests for compactness of the soil were made by counting the number of times a weighted ram had to be dropped from a specified height in order that a conical pin be driven a given distance in the soil (fig. 2). Fifteen determinations of this character were made in each plot and the average of these taken as representative.

The first observations were made on June 19, 1912. The soil was very friable at this time. Several showers had fallen since planting time, and consequently the plots were in excellent tilth.

A definite system was followed in locating places for compactness determinations, similar to the plan for taking samples for analysis. This eliminated any chance of duplicating a measurement of a given spot at later times. Tests were made at least 18 inches apart to avoid further

any influence due to overlapping. In manipulating the mechanical device (fig. 2) auger plate E was placed squarely on the ground and pin D was set in the aperture. Sheath F was then slipped over pin D, and ram G was dropped on the pin until it was driven into the soil sufficiently deep for mark *b* on the ram to be even with the top of sheath F. The ram was raised each time to mark *a* and then dropped freely by its own weight (7,445 gm.). This operation was repeated, recording each drop, until mark *c* on the ram was even with the top of sheath F. Thus, the pin was driven a distance of $4\frac{1}{2}$ inches in the ground each time a test was made. The number of drops necessary to produce this effect was the measure of the relative compactness of soil in the various plots. The results of these trials are given in Table I.

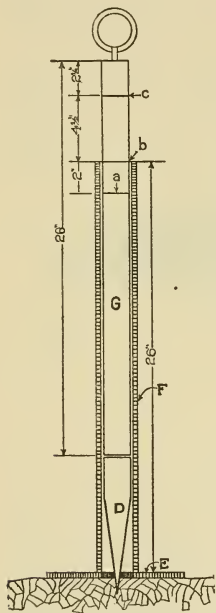


FIG. 2.—Device for testing the compactness of the soil.

The fluctuation between the readings as seen in Table I can not be accounted for other than that it represents the normal variation of soil friability over large areas. Increasing the number of readings did not materially alter the average secured. Therefore, the authentic average compactness of the plowed and that of the unplowed plots stand in the ratio of 1 to 4

at this time. Moisture determinations were made on the following day, with no rain intervening, and were as follows: All plots—first foot, 26.2 per cent; second foot, 26.5 per cent; third foot, 29.3 per cent.

On June 24 all plots were lightly cultivated with a hoe, in order to remove the weeds which had begun to appear. At this time the cowpeas were doing very well and stood about 4 inches high. Samples for nitrate analysis showed the soil to contain at the beginning of the experiment the amounts given in Table II.

As might naturally be expected, there is most nitric nitrogen in the surface foot, with a gradual decrease downward. The analysis of indi-

vidual cores also substantiates the conclusion derived from preliminary tests, that a thoroughly mixed composite is an authentic measure of the actual nitric nitrogen in the soil.

TABLE I.—*Relative compactness (number of drops of ram) of soil on the various plots at the beginning of the experiment (June 19, 1912)*

Trial No.	Plot D (unplowed; clean).	Plot E (unplowed; cowpeas).	Plot F (plowed; cowpeas).	Plot G (plowed; artificial shade).	Plot H (plowed; clean).
1.....	17	8	3	2	3
2.....	22	7	6	3	3
3.....	18	9	3	2	4
4.....	12	8	3	4	3
5.....	13	14	3	4	3
6.....	12	12	3	3	2
7.....	12	13	4	3	4
8.....	10	11	6	3	2
9.....	13	9	3	4	2
10.....	11	13	4	3	3
11.....	10	15	3	4	2
12.....	12	10	3	3	3
13.....	16	9	6	5	1
14.....	11	7	3	5	2
15.....	12	7	5	4	2
Average.....	13.3	10.5	3.6	3.4	3.6

TABLE II.—*Quantity of nitrate as NO₃ in the soil of all plots (June 24, 1912)^a*

No. of core.	Quantity of nitrate (p. p. m.)—		
	First foot.	Second foot.	Third foot.
13 C.....	6.14	3.21	5.11
14 C.....	6.93	6.13	2.37
15 C.....	6.46	3.51	3.27
16 C.....	7.26	3.20	3.66
17 C.....	12.25	3.76	3.05
18 C.....	3.93	3.25	4.78
19 C.....	9.15	4.05	2.09
20 C.....	5.86	3.69	4.35
21 C.....	7.43	3.37	2.26
22 C.....	9.30	3.76	2.58
Average.....	7.46	3.79	3.35
Composite.....	8.06	3.81	3.56
Final.....	7.76	3.80	3.45

Plate XLII shows the general plan of the experiment and the thriftiness of the cowpeas at the early date of July 17—about a month after planting the cowpeas.

^a The nitrate determinations were made by using the phenoldisulphonic-acid method, as suggested by Schreiner, Oswald, and Failyer, George H., in *Colorimetric, turbidity, and titration methods used in soil investigations*. U. S. Dept. Agr. Bur. Soils Bul. 31, p. 39-41, 1906.

Observations taken on August 21 showed that the cowpeas on the plowed plot were only a little heavier than those on the adjacent unplowed plot. Blossoms had already begun to appear, and runners measured from 1 to 2 feet in length. Some crab-grass had sprung up, but only a few other weeds were noticed. The shade devices were in very good condition and the soil beneath seemed normal except that it was covered with a growth of green algæ. This was also true of the soil of the cowpea plots, but to a less marked extent.

Great care was given to details, such as freeing from weeds, renewing the covering of the shade device, etc., throughout the season. Just before frost, compactness tests were again made on all plots after removing the cowpea vines. The vines were cut with a scythe and the strip walked on by the operator was eliminated from the test areas. The data on soil compactness secured for October 15 are given in Table III.

TABLE III.—*Relative compactness (number of drops of ram) of soil on the various plots, as measured on October 15, 1912*

Trial No.	Plot D (unplowed; clean).	Plot E (unplowed; cowpeas).	Plot F (plowed; cowpeas).	Plot G (plowed; arti- ficial shade).	Plot H (plowed; clean).
1	20	18	4	6	5
2	19	12	5	6	6
3	17	19	3	5	7
4	18	11	3	6	6
5	20	14	5	6	6
6	24	17	3	6	5
7	20	14	4	6	5
8	20	15	3	5	5
9	19	17	3	5	7
10	22	17	5	5	6
11	16	15	4	7	8
12	16	15	5	6	5
13	23	15	3	6	5
14	19	3	4	7	6
15	19	18	5	7	5
16	20	16	3	6	7
17	21	16	4	6	7
18	18	15	5	6	8
19	19	11	5	5	6
Average	19.4	15.4	4	5.9	6

The relative compactness as shown in Table III was duplicated, using a modification of the method which originated with Wollny ¹—i. e., the apparent specific gravity of the soil in each plot was determined. A metallic brass tube 7.8 cm. in diameter was driven to a depth of 23.2 cm. in the soil. The tube was then dug out and the contact below broken. Duplicate cores of soil from each plot were thus secured, taken to the laboratory, dried, and weighed. The dry weight of the soil divided by the volume of the cylinder (1,465 c. c.) is the apparent specific gravity

¹ Wollny, Ewald. Der Einfluss der Pflanzendecke und Beschattung . . . 197 p., 10 pl. Berlin, 1877.

and should be an index to friability (Table IV). Wollny compared the porosity of cores similarly taken by measuring the relative amounts of water needed to fill the pore space, but the principle is the same in both cases.

TABLE IV.—*Apparent specific gravity of soil under various treatments as determined on October 15, 1912*

Plot No. and treatment.	Weight of soil.		Average weight of core.	Apparent specific gravity.
	Core No. 1.	Core No. 2.		
	Gm.	Gm.	Gm.	
D (unplowed; clean)	1,957	1,936	1,946	1.33
E (unplowed; cowpeas)	1,865	1,884	1,884	1.26
F (plowed; cowpeas)	1,720	1,739	1,729	1.17
G (plowed; shade)	1,740	1,752	1,746	1.18
G (plowed; clean)	1,635	1,742	1,756	1.19

Checking the results found by the Wollny method with those shown in Table III, the same ratio is found to hold in every case. This gives strong assurance that the use of the compactness device, by means of which the results of Table III were obtained, is an accurate method of measuring soil friability, and, in that it is easily and rapidly made, a very desirable one.

TABLE V.—*Percentage of moisture in the various experimental plots on October 15, 1912*

Plot No. and treatment.	Percentage of moisture.			
	First foot.	Second foot.	Third foot.	Fourth foot.
D (unplowed; clean)	17.9	29.4	24.2	22.5
E (unplowed; cowpeas)	25.2	28.1	17.9	13.6
F (plowed; cowpeas)	21.7	26.1	16.5	18.8
G (plowed; shade)	19.2	29.0	25.9	26.9
H (plowed; clean)	11.2	28.3	27.9	25.3

A study of the moisture in the soil at the close of the experiment, as shown by Table V, reveals, as would be expected, that the plots in cowpeas leave less moisture in the soil than do the uncropped plots kept clean. However, this use of water is from below the second foot. Under cowpeas the surface foot, as well as the second foot, contains as much water as is found in the uncropped plots for the same depth. It would seem, then, that the cowpea plant is a comparatively deep feeder and the shade of its leaves serves as a blanket to prevent evaporation. This conclusion is again borne out by a study of the moisture content of the soil under the artificial shade.

Now, since only the moisture in the first foot could possibly affect the degree of compactness or of looseness at any one time, a direct comparison

of the data given in Table III with those secured at the beginning of the experiment (Table I) can be made, for on October 15 the moisture in the first foot of every plot except H was within the limit of variation, where by preliminary tests the effects due to water can be appreciated by our means of measurement. Therefore, disregarding water as a factor, it is apparent that cowpeas possibly have a tendency to maintain the friability of either plowed or unplowed land. The data also show that the plot G, plowed and artificially shaded, was almost as compact as the adjoining plowed plot (H) which was not shaded. This may be interpreted either that the shade was inefficient or that the loosening of the soil is due to some other factor. From the conclusions of Wollny¹ on this point and from the experimental data to be presented below it seems probable that this preservation of soil structure is due to increased bacterial activity, resulting in the formation of humus. This was actually demonstrated by Wollny.

The nitrate analysis of the plots at the close of the experiment, together with the bacterial count and the nitrifying and ammonifying efficiency, is given in Table VI.

TABLE VI.—Nitrate analysis, bacterial count, and nitrifying and ammonifying efficiency of soil on October 15, 1912

Item.	Depth.	Plot D (unplowed; clean).	Plot E (unplowed; cowpeas).	Plot F (plowed; cowpeas).	Plot G (plowed; shaded).	Plot H (plowed; clean).
Nitrate as NO ₃ ...p. p. m..	First foot...	16. 93	9. 76	17. 833	5. 06	40. 91
	Second foot	5. 88	4. 42	7. 08	11. 55	10. 30
	Third foot..	6. 31	9. 18	4. 08	18. 42	10. 20
	Fourth foot	4. 42	3. 73	4. 48	4. 72	7. 69
Number of bac- teria per gram of soil.	First foot..	8, 481, 000	29, 985, 000	17, 929, 000	9, 344, 400	7, 720, 000
Ammonifying efficiency. ^ado.....	197. 19	166. 20	177. 50	163. 80	167. 20
Nitrifying effi- ciency.do.....	73. 50	65. 40	99. 25	124. 25	—5. 50

^a The determination of ammonia in the ammonifying-efficiency studies was made by the distillation and titration method.

The amounts of nitric nitrogen in the soil in the fall, as shown by the data of Table VI, reveal the fact that all plots are going into winter with more available nitrogen in the soil than they contained in the early spring, as shown in Table II. It is also seen that cultivated plots, either cropped or uncropped, are richer in nitric nitrogen at the end of the season than are the plots not plowed. The low nitrate content of the first foot of the plot artificially shaded can not be explained. Lastly, the results check with previous investigations in the fact that under even a legume treatment there exists less nitrate in the soil in the fall than

¹ Wollny, Ewald. Op. cit.

under adjacent, similarly treated, fallowed plots. (See "Historical summary.")

Although there is a wide range in the total bacterial count under the respective treatments, the only certain conclusion which can be drawn is that under cowpeas we have larger numbers of bacteria than where no crop is on the land. The ammonifying and nitrifying efficiency of these soils as affected by the summer's treatment seemed to have been only influenced by the varied conditions noted, but no correlations can be drawn. Thus, briefly summing up, it might be said that the maintenance of soil structure from spring to fall by the growth of cowpeas on the land is due partially to the shading effect of the foliage, which, like the artificial shade, resists the compacting effect of beating rains and baking sun. Besides this, there seems to be a marked correlation between the friability of the soil under cowpeas and the bacterial flora present. Where present in largest numbers, they possibly bring about a greater production of active humus and so maintain the looseness of the soil.

SUMMARY

(1) The data given show conclusively that cowpeas tend to maintain the friability of loose and compact seed beds.

(2) It was also noted that, while cowpeas take more water from the soil than evaporates from uncultivated adjacent lands, the removal of water is from below the second foot of soil.

(3) Land that was plowed and left uncultivated or plowed and seeded to cowpeas contained a greater quantity of nitrates in the soil at the end of the season than unplowed land similarly treated.

(4) The bacterial activities of the soil upon which cowpeas were grown tended to show that the soil organisms are probably a factor in preventing the packing of soil, as also is the mechanical shade effect of the crop grown upon the land.

PLATE XLII

Experimental plots at Missouri Experiment Station:

Fig. 1.—Plot D (right), unplowed, no crop, kept clean; plot E (center), unplowed, planted to cowpeas; plot F (left), plowed, planted to cowpeas.

Fig. 2.—Plot G (right), plowed, no crop, artificially shaded; plot H (left), plowed, no crop, kept clean.



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TRANSLOCATION OF MINERAL CONSTITUENTS OF SEEDS AND TUBERS OF CERTAIN PLANTS DURING GROWTH

By G. DAVIS BUCKNER,¹

Chemist, Kentucky Agricultural Experiment Station

INTRODUCTION

Several years ago it was observed by Dr. J. H. Kastle, Director of the Kentucky Experiment Station, that the morning-glory vine (*Ipomoea purpurea*) after removal from the soil would continue to grow when its roots were immersed in rain water. Often the growth of this vine attained a length of several feet, bloomed, and produced seeds. During this period the lower leaves etiolated, withered, and ultimately dried up. Evidently the new growth attained by this plant under these conditions was largely at the expense of the various materials contained in the roots, the lower part of the stem, and the lower leaves; especially was this true of the mineral matter required by the new growth, inasmuch as no mineral substance was supplied by the rain water. It therefore occurred to Dr. Kastle that it would be of interest to determine the translocation of the mineral matter in this vine under these conditions. Accordingly, a number of morning-glory vines were completely removed from the soil in which they had grown, and the soil was carefully washed from their roots, which were placed in wide-mouth bottles containing distilled water, the vines being trained on strings arranged vertically in a window. Under these circumstances the vines were found to increase in length by several feet. They put out new roots and a large number of new leaves and in many instances bloomed and produced seeds. Unfortunately, with the limited space at our disposal we were unable to secure a sufficient amount of material to determine the translocation of the mineral substances of the plants under these conditions, and it was found necessary to abandon the experiment with the morning-glory for the time being. However,

¹ The writer wishes to acknowledge the many valuable suggestions made by Dr. Kastle during the progress of these experiments.

we are still of the opinion that on account of its hardness under all sorts of conditions this plant would lend itself better than any other to such studies as those herein contemplated, and we hope to take it up again at some future time.

In thinking over the subject of the translocation of mineral matter during plant growth it occurred to us that it might be of interest to determine the translocation of the mineral matter contained in the seeds and tubers of certain plants during the period of sprouting. Therefore, our present experiments have been confined to the seeds of the garden bean (*Phaseolus vulgaris*), corn (*Zea mays*), and to the potato tuber (*Solanum tuberosum*). Up to this time our work has been confined to the measurement of the translocation of phosphorus, calcium, potassium, magnesium, and silicon.

EXPERIMENTS WITH GARDEN BEANS

The cotyledons of the garden bean were found to contain a considerable amount of mineral matter, and the seedlings of this plant are hardy and well adapted to our requirements. The only difficulties experienced in growing these seedlings under the conditions of these experiments were the growth of molds and the attack of the seedling by the damping-off wilt. The bean in this instance was germinated and allowed to grow to maturity at the expense of the food stored in the cotyledons, extreme care being taken that they should receive no mineral food from external sources. We, of course, realized that the growth of any plant in distilled water is more or less abnormal; yet these beans germinated and produced perfect seedlings with well-developed leaves.

Great difficulty was experienced in keeping down the growth of molds during the process of germination and in preventing the damping-off wilt from attacking the seedlings. In order to overcome these difficulties, every precaution was taken to sprout and grow these seedlings under aseptic conditions. The distilled water employed was boiled for 20 minutes before coming in contact with the beans. The germination and growth of the seedlings were carried out in a dust-proof closet constructed for that purpose. A framework of wood was made and covered inside and out with cheesecloth, leaving an air space of about 2 inches. During the experiment both layers of the cheesecloth were kept moistened with a 50 per cent solution of glycerin and water. This prevented dust and spores from entering the closet; yet it allowed a free passage of air and light. An opening was made in the side of the closet just large enough to admit the head and shoulders of a man. Over this opening was hung a curtain, so arranged as to exclude dust while working inside and when the closet was closed.

The seedlings were never allowed to come in contact with glass. The germinations were made in large porcelain evaporating dishes in which

were placed round perforated porcelain plates, similar to those used in desiccators, on top of which were placed two circular pieces of blotting paper which had been treated with dilute hydrochloric acid and washed free from chlorids with distilled water. Small lamp wicks connected these blotters with the water in the bottom of the dish, so that they would remain moist during the period of germination. Just previous to placing the beans between the blotters the entire apparatus was sterilized by heating at 180° C. for two hours.

The germinated beans were transplanted to test tubes which had been carefully paraffined inside and in each of which was placed a plug of cotton about half an inch from the top and held in place by a small amount of paraffin. The cotton was the purest we could obtain and was treated with dilute hydrochloric acid and washed with sterile distilled water until no test for chlorids could be obtained. This cotton gave practically no ash when incinerated.

In beginning this experiment 1,400 perfect beans were selected, cleaned with a damp cloth, and divided into two lots of 700 each. These lots were labeled "A" and "B," respectively. The 700 beans labeled "A" were placed in a flask and covered with 95 per cent alcohol containing 20 per cent of formalin and allowed to stand for 20 minutes. The beans were then drained and washed free from alcohol with sterile distilled water. The alcoholic drainage and washings were evaporated to dryness and saved for analysis, being labeled "11" in Table I. The beans were now transferred to the sterile germinating dishes described above and placed between blotters, care being taken that the beans did not touch each other. Throughout the germination of the beans sterile distilled water was added in just sufficient amounts to keep the beans moist. Germination started at once, and the small radicle appeared in from two to three days and in some instances was half an inch in length by the end of the fourth day. As soon as this stage was reached, the integuments were removed from the cotyledons with sterile, platinum-tipped forceps, care being taken not to bruise the cotyledons nor allow dust or dirt to come in contact with them. The integuments were preserved and labeled "9" in Table I. The seedlings were then transferred to paraffined test tubes $\frac{3}{4}$ by 6 inches, the seedlings being held in place with a small quantity of sterile cotton. The test tubes were filled with sterile distilled water, which was replaced as fast as it was removed by the plant or by evaporation. The seedlings began to grow immediately, putting forth roots and plumules. Some of the beans on germinating proved to have imperfect cotyledons; these with a number which had been bruised during the removal of the integuments were discarded, so that at the end of the experiment only 609 seedlings had been allowed to mature. This number furnished the material for analysis.

TABLE I.—Analysis of separate parts of bean seedlings and whole beans

(A) SEEDLINGS

Part.	No. of part.	Total weight of air-dried material.	Total weight of ash.	Ash in air-dried material.	Phosphorus as P_2O_5 in ash.		Calcium oxide (CaO) in ash.		Magnesium oxide (MgO) in ash.		Potassium as K_2O in ash.		Silica (SiO_2) in ash.	
					Gm.	Per ct.	Gm.	Per ct.	Gm.	Per ct.	Gm.	Per ct.	Gm.	Per ct.
1, 218 Cotyledons (exhausted)...	7	70.8239	3.3441	4.72	1.1768	35.18	0.0819	2.45	0.0959	2.87	1.5459	46.52	0.0367	1.10
609 Integuments (removed)...	9	16.5423	.6372	3.85	.0121	1.91	.1965	30.85	.0409	7.37	.1224	19.22	.0137	2.00
609 Roots.....	8	7.5516	.7785	10.31	.1916	24.61	.0206	2.65	.0129	1.67	.2903	38.46	.0175	2.25
609 Upper stems.....	6	7.1577	.5611	7.83	.1441	25.69	.0060	1.07	.0097	1.74	.2084	37.16	.0062	1.10
609 Lower stems.....	5	21.6122	1.1339	5.24	.3740	32.99	.0278	2.45	.0341	3.01	.4224	37.26	.0085	.75
1, 218 Leaves.....	4	31.1819	2.1048	6.78	.0664	29.00	.0157	.95	.0374	2.73	.9539	45.32	.0210	1.00
Drain.....	11	3.9526	1.2335	31.20	.2020	18.12	.0242	1.96	.0628	5.09	.5906	48.61	.0102	.82
Total weight.....	158.8222	9.7931	2.707637273197	4.15191138

(B) CONTROL BEANS

1, 218 Whole cotyledons.....	3	169.3022	7.3058	4.31	2.5000	34.22	0.0049	1.30	0.2388	3.27	3.7038	49.19	0.0403	0.55
609 Integuments.....	2	16.4422	.7369	4.48	.0251	3.41	.2505	34.00	.0244	5.76	.1786	24.24	.0081	1.10
Drain.....	1	2.7616	.6354	23.07	.0582	9.16	.0176	2.77	.0237	3.74	.2853	44.44	.0117	1.85
Total weight.....	188.5060	8.6781	2.583336203049	4.16470621

As the growth of the seedlings proceeded, the cotyledons began to shrink and finally turned brown. The root development in all cases was good, nearly filling the test tubes, and each seedling developed two perfect leaves. The seedlings were allowed to grow until they began to etiolate and wilt, this period being reached in from 17 to 22 days. The plants thus grown were very uniform in size and development, the average height being $6\frac{1}{2}$ inches. During their development care was taken that they should not touch each other. As fast as they matured, they were removed from the test tubes and the cotton carefully removed from the stem and roots. The plants were then divided into roots (8),¹ lower stems (5) which averaged $4\frac{1}{2}$ inches in height, exhausted cotyledons (7), upper stems (6) which averaged 2 inches, and the leaves (4). The liquid remaining in the test tubes was evaporated to dryness and added to the washings (11).

Six hundred and nine selected beans labeled "B" received the same treatment as those labeled "A," except they were allowed to live only until the radicle had appeared and the integument had softened. The integument (2) and the cotyledons (3) were carefully air-dried, as were the above-mentioned plants. The drainage and washings (1) from these beans were carefully evaporated to dryness. These several parts of the beans were analyzed to check the analyses of the seedlings, the results of which are given in Table I.

In analyzing the separate portions of the air-dried material which had been carefully ashed at a dull-red heat, three portions of 0.2000 gm. each were carefully weighed out. In one portion phosphorus and silica were determined, while in another portion the determination of potassium was made. The methods used were essentially the official methods of the Association of Official Agricultural Chemists.² In a third portion of the ash, calcium and magnesium were determined according to the method of McCrudden.³

In Table I are to be found the results of the analyses of the separate portions of 609 seedlings and the separate parts of 609 beans.

It is evident from the results given in Table I that the weight of the total ash of the seedlings agrees fairly well with the total weight of the ash of the bean control, the difference being due in all probability to unavoidable outside contamination during the period of growth. The comparative analyses of the inorganic constituents fall well within the limit of experimental error. The greatest difference is observed in the case of silica, the seedlings containing nearly twice as much as the beans.

¹ The numbers in parentheses refer to the number of part in the tables.

² Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908.

³ McCrudden, F. H. The quantitative separation of calcium and magnesium in the presence of phosphates and small amounts of iron devised especially for the analysis of foods, urine, and feces. *In Jour. Biol. Chem.*, v. 7, no. 2, p. 83-100. 1910.

— The determination of calcium in the presence of magnesium and phosphates: the determination of calcium in urine. *In Jour. Biol. Chem.*, v. 10, no. 3, p. 187-199. 1911.

This is probably due to unavoidable contamination. It is of interest to note that the integument contains 52.72 per cent of the total calcium oxid found in the bean; it is also interesting to find that the amount of phosphorus and potassium in the integument is very small. It is shown that a marked accumulation of the mineral elements in the leaves and lower stems occurs during growth. This is more clearly shown where the results are expressed as the percentage distribution of the mineral constituents that actually migrated from the cotyledons, as seen in Table II.

TABLE II.—Percentage distribution of the mineral constituents of bean seedlings

Part.	Part No.	Phosphorus as P_2O_5 .	Calcium oxid (CaO).	Magnesium oxid (MgO).	Potassium as K_2O .	Silica (SiO_2)
Cotyledons (exhausted).....	1	47.20	54.53	45.67	45.67	40.82
Roots.....	4	7.68	13.72	6.14	8.72	19.47
Upper stems.....	5	5.78	3.99	4.62	6.07	6.90
Lower stems.....	3	15.00	18.51	16.24	12.31	9.45
Leaves.....	2	24.34	10.45	27.33	27.83	23.46

In the foregoing experiment we have germinated beans, and they have grown until they died from the want of nourishment. From all physical appearances the growth of the seedlings has been normal. This growth has been at the expense of the food material stored in the cotyledons, the carbon dioxid inspired from the air, and the distilled water received through the roots. Every precaution was taken to exclude all mineral matter from external sources. Referring to Table II, it is seen that approximately 50 per cent of the total mineral content of the cotyledons remained unused and that approximately 50 per cent was translocated to different parts of the seedlings during growth. As might be expected, the greatest quantity of these elements migrate to the leaves and the next greatest quantity locate in the lower stems. The large amount of calcium and silica locating in the roots is also of interest.

These results serve to emphasize the importance of the mineral matter both to the seedlings and to the sprouting seed or cotyledon. In other words, it would seem from these results that the mineral matter originally present in the seed or in the cotyledons functions in the act of sprouting in two different ways: First, to promote the enzymic changes occurring in the sprouting cotyledons and seeds themselves; and, in the second place, to support the growth and development of the seedlings. The growth will therefore depend somewhat at least on the total mineral matter originally present in the cotyledons or seeds, a part of this being translocated to meet the requirements of the growing seedling. Approximately an equal part or, at any rate, a relatively large amount of the mineral matter remains in the seed or cotyledon to support and promote those enzymic changes characteristic of the seed or cotyledon in an active katabolic condition.

EXPERIMENTS WITH CORN

Similar experiments have been tried with corn, except that the seedlings were grown in aluminum cups instead of in paraffined tubes. One thousand grains of corn were germinated, transferred to aluminum cups, and allowed to grow for 23 days, when they began to etiolate. During this time these seedlings attained a height of 9 inches. At this point they were removed from the cups and dissected as follows: Leaves (2), exhausted cotyledons (3), stems (4), and roots (5). (See Table III.) These were controlled by the same number of whole corn grains (1) as given also in Table III. These several lots of material were analyzed in the same manner as the bean seedlings. In this experiment we have also followed the translocation of iron and aluminum. Unfortunately, the results obtained with these two last-named elements show contamination from the aluminum cups used in the experiment. The results of the analyses of the ash of corn grain and of the several parts of the seedlings thereof are given in Table III.

It will be seen from the results of these analyses that the sum of the total ash of the several parts of the corn seedling exceeds the total ash of the corn grain by 0.9487 gm. This is doubtless to be explained by the fact that iron and aluminum were taken up in considerable amounts from the cups and also by contamination with small amounts of dust from the outside air. It will be seen that the sum of the amounts of phosphoric acid, potash, and magnesia in the several parts of the corn seedling agrees with that of the corresponding amounts of these substances found in the corn grain, within the limits of experimental error. A point of interest in this connection is that magnesia is greatly in excess of lime in the grain of corn and in the several parts of the seedling obtained therefrom. The amounts of lime, silicon, iron, or aluminum found in the several parts of the seedling are in excess of the amounts of these substances found in the grain. As already pointed out, this discrepancy is doubtless due to outside contamination. Under the conditions prevailing in this experiment approximately two-thirds of the total mineral matter of the corn grain has been translocated to the stems, roots, and leaves of the seedling during the process of growth. It is evident further that approximately the same amounts of this mineral matter go to stem and roots, respectively, whereas a somewhat larger amount of the mineral matter migrates to the leaves of the seedlings. The fact that a relatively large amount of the mineral matter, amounting in this case to something over one-third of the whole, remains in the exhausted cotyledon is of interest and doubtless has the same significance for the growth of the seedling as is believed to obtain in the case of the bean, already discussed. The percentage distribution of the mineral constituents of corn during the growth of the seedling is shown in Table IV.

TABLE III.—Analysis of separate parts of corn seedlings and whole corn grains

SEEDLINGS																	
Part.	No. of part.	Total weight of air-dried material.	Total weight of ash.	Ash in air-dried material.	Phosphorous as P ₂ O ₅ in ash.		Calcium oxid (CaO) in ash.	Magnesium oxid (MgO) in ash.	Potassium as K ₂ O in ash.	Silica (SiO ₂) in ash.	Iron oxid (Fe ₂ O ₃) in ash.		Aluminum oxid (Al ₂ O ₃) in ash.				
					Gm.	P. ct.					Gm.	P. ct.		Gm.	P. ct.	Gm.	P. ct.
3,000 Leaves.....	2	43.2716	2.0107	4.64	0.7845	39.02	0.0502	2.50	0.1425	7.09	0.6428	31.92	0.0613	3.05	0.0012	0.0596	0.48
1,000 Cotyledons (exhausted).....	3	342.3911	2.7254	7.79	1.2858	47.18	0.0735	2.70	2.335	8.57	3.597	13.20	0.1812	6.65	0.0234	0.0136	0.80
1,000 Stems.....	4	36.7137	1.3292	3.62	0.5186	39.02	0.0221	1.65	0.0835	6.29	0.3808	28.65	0.0198	1.50	0.0107	0.0086	0.65
1,000 Roots.....	5	48.9321	1.3514	2.70	0.3849	28.46	0.0216	1.60	0.0369	2.73	0.4153	29.92	0.0466	3.45	0.0120	0.2471	18.29
Total.....	471.3685	7.4167	2.9738	0.1674	4.964	1.7986	0.3089	0.0473	0.2789
WHOLE GRAINS																	
1,000 Whole corn.....	1	461.9412	6.4680	1.40	3.0250	46.77	0.0562	0.87	0.4760	7.36	1.8919	29.25	0.0822	1.28	0.0116	0.0051	0.079

TABLE IV.—Percentage distribution of the mineral constituents of corn seedlings

Part,	Phosphorus as P ₂ O ₅ .	Calcium oxid (CaO).	Magnesium oxid (MgO).	Potassium as K ₂ O.	Silica (SiO ₂).	Iron oxid (Fe ₂ O ₃).	Aluminum oxid (Al ₂ O ₃).
Leaves.....	26. 38	30. 02	28. 71	35. 74	19. 84	2. 54	3. 44
Exhausted cotyledons.....	43. 24	43. 86	47. 04	20. 00	58. 66	49. 47	4. 88
Stems.....	17. 44	13. 21	16. 82	21. 17	6. 41	22. 62	3. 08
Roots.....	12. 94	12. 91	7. 43	23. 09	15. 09	25. 37	88. 60

A comparison of these individual mineral constituents shows that except in the case of potash approximately 50 per cent thereof have been translocated from the cotyledons to the several parts of the seedling during growth, and that the translocation of potash is greatly in excess of that of the other mineral constituents. The taking up of such considerable amounts of iron and aluminum from the aluminum cups in which these seedlings had been grown and the great accumulation of aluminum on the roots of the seedlings is also a matter of interest, although it has no immediate bearing on the subject under consideration. It is also evident from the foregoing that there is a decided accumulation of translocated mineral matter in the leaves of the seedling, a fact that is in harmony with the rapid growth of the leaves as compared with that of the other parts of the seedling.

EXPERIMENTS WITH POTATOES

In the experiment with the potato tuber we have allowed potatoes to sprout in a dark closet, after they had been thoroughly cleaned. When the tubers began to soften they were removed from the dark closet and the sprouts (1) cut off. The potato was then carefully pared and the skin (2) and the starchy tissue (3) were carefully dried and ashed and the quantities of calcium, magnesium, phosphorus, potassium, and silicon were determined. The results are shown in Table V.

TABLE V.—*Analysis of separate parts of sprouted potatoes*

Part.	Part No.	Total weight of air-dried material.			Total weight of ash.			Ash in air-dried solids.			Phosphorus as P_2O_5 in ash.			Calcium oxid (CaO) in ash.			Magnesium oxid (MgO) in ash.			Potassium as K_2O in ash.			Silica (SiO_2) in ash.		
		Gm.	Gm.	Perc't.	Gm.	Perc't.	Gm.	Perc't.	Gm.	Perc't.	Gm.	Perc't.	Gm.	Perc't.	Gm.	Perc't.	Gm.	Perc't.	Gm.	Perc't.	Gm.	Perc't.	Gm.	Perc't.	
New sprouts.	1	21.3722	2.1185	9.91	0.2661	12.56	0.0190	0.90	0.0443	2.72	0.8558	40.40	0.0201	0.93											
Skin.....	2	46.9359	3.8205	8.14	0.2250	5.89	0.0649	1.70	0.0518	1.33	1.5408	40.33	0.3228	8.45											
Tubers (exhausted)...	3	185.3766	8.1120	4.37	1.0058	12.40	0.0608	0.75	0.1835	2.25	4.3415	53.52	0.0486	0.60											
Total weight.....		253.6847	14.0510		1.4969		1.447		2.796		6.7381		3.915												

The relatively high percentage of ash in the sprout of the potato as compared with that contained in the exhausted tuber is a matter of interest. It will be seen, however, that considerable amounts of ash still remain in the exhausted tuber after the growth of the sprouts is complete, indicating the necessity of mineral matter for those changes occurring in the tuber during the act of sprouting. Table VI gives the percentage distribution of the several mineral constituents between the sprouts and exhausted tubers, including the skin.

TABLE VI.—*Percentage distribution of the mineral constituents of potatoes*

Part.	Phosphorus as P_2O_5 .	Calcium oxid (CaO).	Magnesium oxid (MgO).	Potassium as K_2O .	Silica (SiO_2).
Sprouts.	17.77	13.12	15.84	12.68	5.13
Tubers (exhausted)	67.13	42.02	65.68	64.43	12.41

In Table VI it is observed that a large amount of the mineral material remains unused in the exhausted tuber of the potato and that approximately only 15 per cent of the different mineral constituents have migrated to the sprouts.

CONCLUSIONS

The most striking fact brought out thus far by these studies on the translocation of the mineral matter of the seed and tuber during the growth of the seedling is the retention of considerable amounts of the mineral matter, varying from 46.66 per cent in the garden bean and 38.66 per cent in corn to 50.33 per cent in the potato tuber in the cotyledons and tuber, respectively. As indicated in the foregoing experiments, this probably finds its explanation in the necessity for definite amounts of the various mineral constituents to promote the katabolic changes occurring in the cotyledon and tuber during sprouting. So far as could be ascertained, there were no very striking differences in the quantities of its several mineral constituents translocated and no marked selective influences shown by the roots, stem, and leaves of the growing seedling for any particular mineral reserve material contained in the seed or tuber. Up to the present time, great difficulty has been experienced in the selection of a suitable container in which to grow these seedlings. This has proved a serious obstacle to this work. It is hoped, however, that this difficulty may be finally overcome and better and more constant results obtained through the use of pure paraffin containers.

FATE AND EFFECT OF ARSENIC APPLIED AS A SPRAY FOR WEEDS

By W. T. McGEORGE,

Chemist, Hawaii Agricultural Experiment Station

INTRODUCTION

In certain districts of Hawaii during the rainy season cultivation is impracticable, because of its bad effect upon the texture of the soil. Yet at times this season is abnormally long and especially favorable to the growth of weeds. Weed control is therefore a very important problem for Hawaiian planters. In experiments at the Hawaii Experiment Station¹ it was found that the most economical means of weed control under such conditions lay in the use of chemical sprays. Careful comparative tests were made of such chemicals as sodium arsenite, ferrous sulphate, carbon bisulphid, etc. Of these, sodium arsenite proved by far the most effective and was recommended for use. Sodium arsenite sprays have now been used in Hawaii for weed eradication for about five years and have proved to be efficient and economical. Such sprays have not only been used to replace hand labor in the fields, but also as a means of ridding grass lands of undesirable plants.

In view of the possible injury to soils and crops as a result of the continued use of such sprays, the Hawaii Experiment Station undertook a study of the fate in the soil of the arsenic so applied and its influence upon plant growth and upon ammonification and nitrification.

EFFECT OF SODIUM ARSENITE ON PLANT GROWTH

Apparently there is little or no immediate danger to crops from the use of sodium arsenite as a spray. In fact, in experiments with millet, buckwheat, and cowpeas grown on three different types of Hawaiian soils it was found that small quantities of arsenic stimulate plant growth. However, analyses of the plants did show that the arsenic is assimilated and that when it is present in the tissues in sufficient concentration death of the plant results.

The most surprising feature of the investigation was the influence on the ammonifying and nitrifying bacteria. In one type of soil ammonification was stimulated even by such excessive amounts as 1 per cent of arsenic (As_2O_3) in the soil. The results as a whole indicate that no fear need be entertained regarding any detrimental influences toward the

¹ Wilcox, E. V. Killing weeds with arsenite of soda. Hawaii Agr. Exp. Sta. Press Bul. 30, 15 p. [1911.]
Krauss, F. G. Suppression of weeds among pineapples by arsenite of soda spray. Hawaii Agr. Exp. Sta. Press Bul. 48, 8 p., 2 fig. 1915.

McGeorge, W. T. The effect of arsenite of soda on the soil. Hawaii Agr. Exp. Sta. Press Bul. 50, 16 p., 3 fig. 1915.

organisms upon which the plants rely for their available nitrogen, provided proper soil texture is maintained.

Furthermore, it was found that in time the arsenic practically loses its toxic influence toward plants. This was shown by the comparative growth of plants on soils treated at time of seeding and those seeded several months following the application of the arsenic to the soil. There are only two possible explanations of this condition: Either the arsenic reacts with certain of the soil constituents, resulting in a less toxic combination, or it is rapidly leached from the soil.

ABSORPTION OF ARSENIC BY THE SOIL

When a soluble salt is added to a soil, its ultimate disposition must depend upon certain chemical reactions and physical phenomena. In this case the possibilities involve (1) a combination with or replacement of salts already present, resulting in its absorption as a whole; or (2) a selective absorption involving the fixation of only one ion of the salt.

In order to determine the fate of arsenic and the effect of irrigation, a set of lysimeter experiments was inaugurated.

LYSIMETER EXPERIMENTS

Three types of soil were selected: (1) A ferruginous red clay, (2) a ferruginous brown clay, and (3) a highly organic silt. Twenty-five pounds of soil were placed in each of six lysimeters, two being filled with each type. To each soil were added 3 liters of a solution of sodium arsenite of the same strength as that used for spraying weeds. One series of three was allowed to stand for two months protected from rain. To the other three 1 liter of water was added every other day for several weeks, after which the soil was allowed to stand in the lysimeter until dry enough to sample.

The object of these experiments was to determine the rate of fixation, the depth to which the arsenic can penetrate, and the leaching effect of irrigation. At the expiration of the above time samples were taken at various depths in the lysimeters and the percentage of arsenic (As_2O_3) in the soil at each depth was determined. The results are given in Table I.

TABLE I.—*Effect of irrigation on arsenic in the soil, giving the percentage of arsenic at various depths*

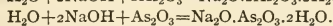
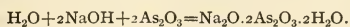
Soil No. 1.			Soil No. 2.			Soil No. 3.		
Depth.	Not irrigated.	Irrigated.	Depth.	Not irrigated.	Irrigated.	Depth.	Not irrigated.	Irrigated.
<i>Inches.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Inches.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Inches.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1 to 3	0.280	0.224	1 to 3	0.450	0.237	1 to 2	0.97	0.95
3 to 5	.198	.211	3 to 5	.170	.092	2 to 4	.50	.47
5 to 7	.171	.145	5 to 7	.118	.092	4 to 6	0	0
7 to 9	.184	.170	7 to 9	.013

The columns headed "Not irrigated" show the percentage of arsenic in the soil at the given depth in the lysimeters which were protected from rain and which received no irrigation. The columns headed "Irrigated" show the percentage of arsenic in the soil at the given depth in the lysimeters which were subjected to irrigation. A comparison of the two columns for each soil will show the strong fixing power of these soils for arsenic, the influence of different soil types upon the fixation, and the danger of its accumulation. Samples of soil No. 3 were taken at depths different from those of soils Nos. 1 and 2, as shown in Table I, because of the concentration of arsenic at the surface in the former.

In order to determine how nearly these results represent actual field conditions, samples of soil were obtained from a plantation at Nahiku, Maui, which was the first to adopt the use of sodium arsenite as a means of weed control. Weeds on this land have been sprayed for five years, at the rate of three applications per year, using 5 pounds of arsenic (As_2O_3) per acre for one application. During this time the soil has received no cultivation whatever and the rainfall averages about 200 inches per year. The soil is very porous and there is very little run-off water. Samples were taken at three depths: Every 4 inches of the first foot. The surface 4 inches contained 0.00924 per cent of arsenic (As_2O_3), and none was present below this depth. A determination made by boiling the soil with water showed an arsenic content of 0.00006 per cent, or 0.6 p. p. m., soluble in water. That the arsenic fixed by soils in the lysimeters was partly soluble in water indicates that the fixation is due in part to physical influences.

CHEMICAL REACTIONS INVOLVED IN THE FIXATION

The composition of the spray as prepared by recommended methods may be either a solution of the acid salt ($\text{Na}_2\text{O} \cdot 2\text{As}_2\text{O}_3 \cdot 2\text{H}_2\text{O}$) or the neutral salt ($\text{Na}_2\text{O} \cdot \text{As}_2\text{O}_3$), depending on the proportions of soda (either hydrate or bicarbonate) and arsenious acid used.



For the following experiments in studying the replacement phenomena, a solution of the neutral salt was used.

One liter of a 1 per cent solution of sodium arsenite was allowed to act upon 200 gm. of soil, with occasional shaking, for two weeks. Checks were also maintained with 200 gm. of soil and 1 liter of water. The arsenic extract was then separated from the soil and a partial analysis made to determine the elements with which the sodium arsenite is most active. The results are given in Table II, which shows the composition of a 1 per cent sodium-arsenite solution after contact with the soil, as compared with the solvent action of water. The percentage of humus

in the soil before and after treating with 1 per cent of sodium arsenite is also given.

TABLE II.—Composition of the extracts (mgm. per liter)

Constituent.	Soil No. 1.		Soil No. 2.		Soil No. 3.	
	Water extract.	Arsenic extract.	Water extract.	Arsenic extract.	Water extract.	Arsenic extract.
Fe ₂ O ₃	Trace.	716	Trace.	121	Trace.	90
CaO.....	11.2	84	13.6	124	74.6	126
MgO.....	3.6	20	10.8	44	7.4	26
As ₂ O ₃		3,960		6,000		4,480
Mg.As ₂ O ₃ fixed by 100 gm. soil.....		2,640		600		2,120
Humus I ^a , per cent.....	2.77		1.68		8.75	
Humus II ^a , per cent.....	1.56		1.80		8.40	

^a Humus I shows the percentage of the humus content of original soil; humus II, that of soil after treatment with the 1 per cent sodium-arsenite solution.

Table II shows a replacement of and a solvent action toward iron, calcium, magnesium, and humus, and suggests several theories as to the nature of the reaction. The soil absorbing the largest amount of arsenic lost through solution or replacement the most iron and humus. The soil absorbing the least arsenic lost the least iron and no humus. Apparently the absorption of arsenic by soil No. 3 is largely a mechanical fixation, as the data show a high absorption, but a low replacement.

In sodium arsenite we have the combination of a strong base with a weak acid. A well-known property of such salts is to react alkaline when dissolved in water. This is due to the faint dissociation of H₂O into H⁺ and OH⁻ ions. Here the chemical and physical phenomena involved in the fixation of sodium arsenite are directly or indirectly a result of hydrolysis. The latter term as used herewith is intended to convey the increased dissociation in a solution of sodium arsenite, which itself is only faintly dissociated. This results in an increase in the concentration of the hydroxyl ion and the formation of the highly dissociated electrolyte sodium hydrate, which in the soil would probably be rapidly converted to bicarbonate. In this form it would have a solvent action toward the iron and humus and more or less toward the magnesium and calcium through the formation of slightly soluble bicarbonates. Magnesium bicarbonate is very unstable as compared to calcium bicarbonate and, hence, is precipitated following the solvent action of the sodium bicarbonate. The calcium is more soluble even in the soils containing much higher amounts of magnesium. These reactions leave the arsenic free as the negative ion to combine with the dibasic and tribasic metals to form slightly soluble arsenites or arsenates, thereby fixing the arsenic in the soil.

The rate and extent of fixation of arsenic vary in different soil types, owing to the concentration and solubility of the basic constituents—i. e., dissociation was found to be more rapid in some soils than others. To illustrate, the soil absorbing the greatest amount of arsenic exhibited the strongest alkalinity and showed the greatest chemical activity. Furthermore, this same soil contained the least amount of the soluble bases, calcium, magnesium, and potassium, indicating that the chemical fixation is influenced by the pressure of soluble bases.

SUMMARY

It has been shown herein that soils possess strong fixing power for arsenic and that when a sodium-arsenite spray is used for destroying weeds the arsenic will ultimately be deposited in the surface soil, there to remain in spite of the leaching effect of rains or irrigation.

The chemical reactions involved in the fixation are a replacement or solution of iron, calcium, magnesium, and humus, owing in part to a hydrolysis of the sodium arsenite in solution, also a combination with the dibasic and tribasic elements to form the difficultly soluble arsenites or arsenates.

ANGULAR LEAF-SPOT OF CUCUMBERS

By ERWIN F. SMITH, *Pathologist in Charge*, and MARY KATHERINE BRYAN, *Scientific Assistant, Laboratory of Plant Pathology, Bureau of Plant Industry*

INTRODUCTION

The angular leaf-spot of cucumbers (*Cucumis sativus*) has been known in the field for many years, but up to the present time no organism has been named as its cause, though it has been generally conceded to be of bacterial origin. The disease is characterized by the formation of numerous, often confluent, angular, dry, brown spots which by dropping out or tearing give the leaves a ragged appearance.

The literature on the subject, aside from mere notes on the occurrence of the disease scattered through pathological literature, consists of four papers by O. F. Burger, of Florida,¹ and a more recent Italian paper by Traverso.² Burger mentions the leaf-spot as preliminary to a more destructive fruit-rot, said to be due to the same organism. His description of the diseased leaves agrees with the appearance of leaves sent to the writers from Wisconsin, as well as with those obtained by them from other States, and with the leaf-spots which they obtained in Washington by pure-culture inoculations. A brief description of the causal organism is given in each of his papers, in one case with the group number according to the chart of the Society of American Bacteriologists. Burger's descriptions agree in the main except as to flagella and the diameter of his organism. In his earlier descriptions it is said to have polar flagella, but in the later ones it is reported to be peritrichiate. No name is given to the bacillus.

Traverso's paper is only a preliminary one, but it leaves no doubt as to the identity of the Italian and American disease. A motile, fluorescent, nonliquefying organism was isolated by him and inoculations were made with it, but no positive results were obtained (p. 459).

Who first reported this cucumber disease in the United States is uncertain; the senior writer has known it for 20 years, and several years ago (1904) plated out two yellow bacteria with which unsuccessful inoculations were made. Again, in 1907, at his suggestion, Mr. John R. Johnston, then of the Laboratory of Plant Pathology, made platings

¹ Burger, O. F. A new cucumber disease. *In* Fla. Agr. Exp. Sta. Rpt. [1911]/12, p. c-cl. 1913.

——— A bacterial rot of cucumbers. *In* Phytopathology, v. 3, no. 3, p. 169-170. 1913.

——— Bacterial rot of cucumbers. *In* Fla. Agr. Exp. Sta. Rpt. [1912]/13, p. xc-xciv, fig. 11-13. 1914.

——— Cucumber rot. Fla. Agr. Exp. Sta. Bul. 121, p. 97-109, fig. 37-42. 1914.

² Traverso, G. B. Sulla batteriosi del cetriolo in Italia. Nota preliminare. *Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat.*, s. 5, v. 24, sem. 1, fasc. 5, p. 456-460. Apr. 5, 1915.

and isolated a yellow schizomycete with which unsuccessful inoculations were made on cucumbers in the Department greenhouses.

ISOLATION AND IDENTIFICATION OF ORGANISM

Specimens were sent to the Laboratory of Plant Pathology in August and September, 1914, from New York and Wisconsin. No complaint was made by the sender of any association with fruit-rot, either on his own initiative or when questioned.

The interior of the spots was found to be swarming with bacteria which on floating out on the slide showed active motility. Plates were poured from such spots and a white, motile, rod-shaped organism was isolated. Spray inoculations with subcultures from three colonies on these plates gave typical infections on young cucumber leaves, from which the organism was reisolated. Colonies (subcultures) from this reisolation were then used for spray inoculations, and again the typical disease was produced with great virulence.

In August, 1915, specimens were received from several localities in Wisconsin, Indiana, and New York and from Ontario, Canada. In each case the same organism was isolated in pure cultures and used to produce typical infections on cucumber leaves in the hothouse.

The organism causing the angular leaf-spot of cucumbers appears to be an undescribed form for which the specific name *lachrymans* is suggested on account of the tearlike drops of exudate from the spots in early stages of the disease. Its brief Latin diagnosis is as follows:

***Bacterium lachrymans*, sp. nov.**

Baculis cylindricis apicibus rotundatis, solitariis, saepe binis; baculis unis $0.8 \times 1-2\mu$; 1-5 flagellis polaribus mobilibus; aerobiis, asporis.

Habitat in foliis vivis Cucumeris sativi in maculis angularibus. Liquefacit gelatinam lente. Colonae superficiales in agar-agar, rotundae, albae; colonae juvenes habientes centra non-translucida, et margines translucidas cum lineis multis radiantibus. Lac sterile alkalinum et translucidum fit; casein non segregatur. Nitrum non redigitur; culturae in mediis cum saccharo sacchari et saccharo uvae acidae fiunt. Gas non facitur. Methodo Grami non coloratur.

The organism which the writers isolated from the Wisconsin cucumber leaves and have here designated "*Bacterium lachrymans*, n. sp." differed culturally in so many important respects from Burger's organism that all our cultural experiments were repeated. These repetitions, however, confirmed the differences, which are given in Table I.

While it is not doubted that Burger had this disease under observation, it is believed that the organism described by him is not its cause, but is rather the cause of a rapid soft-rot of the fruit. His organism, however, may be a wound parasite following injuries due to the organism here described.

TABLE I.—*Differences between Bacterium lachrymans and Burger's cucumber organism*

<i>Bacterium lachrymans.</i>	Burger's organism.
1. Polar flagellate.....	Peritrichiate flagellate.
2. Liquefies gelatin.....	Does not liquefy gelatin.
3. Clears milk without coagulation.....	Coagulates milk.
4. Strict aerobe (does not grow in closed end of fermentation tubes).	Facultative anaerobe (grows in closed end of fermentation tubes).
5. Forms acid from saccharose in fermentation tubes.	Does not form acid from saccharose in fermentation tubes.
6. Forms acid from dextrose in fermentation tubes.	Does not form acid from dextrose in fermentation tubes.
7. Not villous along line of stab in either agar or gelatin.	Villous along line of stab in both gelatin and agar.
8. Does not become yellow with age on sugar agars.	Becomes yellow with age on sugar agars.
9. Moderate indol formation.....	No indol formation.
10. Agar-plate surface colonies show many fine radiating lines.	Agar-plate colonies homogeneous in structure.
11. Does not cause soft-rot of cucumber fruits.	Causes a soft-rot of the fruit.
12. Surface colonies on agar plates are always round.	Agar colonies are round to ameboid.

GEOGRAPHICAL DISTRIBUTION OF THE DISEASE

Mr. Frederick V. Rand, of this laboratory, by whom these specimens were collected, reported the disease in 1915, from the following localities:

MICHIGAN: Big Rapids, Muskegon, Grand Haven, Holland, Grand Rapids, and Hudsonville.

INDIANA: Plymouth, Monterey, Tyner, and Donaldson.

WISCONSIN: Racine, Portage, Ripon, Princeton, and Milwaukee.

NEW YORK: Constable, Malone, North Lawrence, and Long Island.

CANADA: Provinces of Ontario and Quebec.

In regard to the amount of injury caused by this disease, Mr. Rand says:

In most cases I found the angular leaf-spot causing a rather minor injury, but in an occasional field I found all the leaves back of the tips of the vines very badly shot-holed and presenting an exceedingly ragged appearance, such that serious injury to the crop must inevitably result. Last year this disease had done more damage than any other in the vicinity of Ripon, Wis.

This disease has also been reported recently from Maryland and several other Southern States.

Earlier the senior writer received specimens from Michigan, Wisconsin, Indiana, Connecticut, and the District of Columbia.

INOCULATION EXPERIMENTS

On October 26, 1914, young cucumber plants were sprayed in cages in the hothouse with water suspensions from young agar slants made from three colonies on the plates poured from diseased leaves. The plants were kept moist in the cages for 30 hours, then removed to the bench.

Five days later, water-soaked spots appeared on the leaves, and by November 3 there were typical browned spots on plants inoculated with each of the three colonies. These spots swarmed with bacteria. Poured plates on agar gave pure cultures of the same white organism. No further inoculations were made until April 30, 1915, when sprayings were again made in cages as before, using subcultures of colony No. 1, plated from a spot produced by the inoculations of October 26. The plants used in this case were of a common field variety and rather stunted but with sound leaves. Three days after the first spraying water-soaked spots appeared on the lower surface of the leaves, and by May 6 these had enlarged into the typical angular, dry, brown spots.

Another experiment on May 6, 1915, using perfectly healthy, free-growing Arlington white spine cucumber plants and subcultures from the same colony (No. 1) gave striking results. Several leaves showed tiny water-soaked areas on the second day, and all the leaves were typically and badly spotted by the sixth or seventh day. In this stage the spots were one-fourth to three-fourths of an inch in diameter, angular, following the larger veins, and water-soaked (translucent), not dry. In the early morning drops of moisture (exudate) swarming with bacteria were found hanging on the lower surface of such spots (Pl. XLV, fig. 1). Pure cultures of the causal organism were obtained by plating from one of these drops. On the following day, or even later on the same day, white films (bacterial crusts) replaced the drops (Pl. XLIII, fig. 1). The appearance of infected leaves at the end of 12 to 14 days, when the diseased areas have become dry and begin to drop out, is shown in Plate XLIII, figure 2.

As the young unsprayed leaves developed on these plants, they became naturally infected; and in three cases the stems and petioles of this young growth also became water-soaked, exuded drops of fluid (Pl. XLIV, X, X), and finally broke or bent over (Pl. XLV, fig. 2), ending the growth of the plant. The cracking open of stems in this stage of the disease is shown at X in Plate XLV, figure 2, and in detail in Plate XLV, figure 3.

On the green fruits up to the end of August, 1915, the writers were able, with one exception, to obtain within a week or 10 days (shipping time) only a local infection and a bacterial exudate such as that shown in Plate XLVI, figure 1—no general soft-rot. Even when the fruit (Pl. XLVI, fig. 1) was kept for another week at high temperatures (28° to 32° C.), it did not rot (Pl. XLVI, fig. 2). Altogether 15 such fruits were inoculated with virulent cultures, some on the vines and others in damp chambers.

Soft-rot occurred twice in young fruits (two-thirds grown) when placed in damp chambers after inoculation. In the first case (the exception referred to above), plates were poured from the soft interior of the one fruit thus affected. As only spreading fimbriate colonies were obtained, the soft-rot was attributed to an intruder, and no further studies were

made. Some months later (September, 1915) in a similar experiment two out of four inoculated fruits became soft-rotted. These fruits were from the market. All four showed the local gumming at the point of inoculation (needle pricks) after five days, while check pricks gave no gumming. Two days later two fruits began to soften, and the next day the whole interior was swarming with bacteria. Plates were poured from the interior of one of these fruits under sterile conditions, and again only spreading fimbriate colonies were obtained. Smears from these colonies stained by Van Ermengem's flagella stain gave rods with as many as 8 or 10 peritrichiate flagella. This organism grew well in the depths of agar stabs and curdled milk with reddening of litmus in milk. The other two inoculated fruits remained sound and after two weeks when cut open showed only a very local infection not extending much beyond the needle pricks in any direction.

Since the organism causing the leaf-spot is polar flagellate and aerobic, does not develop a fimbriate growth on agar, and does not curdle milk or redden litmus in milk, it is evident that this soft-rot was due to an intruder, which may have come from the surface of the fruits, since they were not sterilized, but only washed.

When these fruits became soft-rotted, the suspicion arose that possibly the softening and cracking of the stems and petioles (Pl. XLV, fig. 2) might also have been due to some unsuspected soft-rot organism. The inoculation experiments with *Bact. lachrymans* were therefore repeated on stems and petioles of free-growing cucumbers with the same result as before—i. e., softening and cracking of the younger stems and petioles. From one of these stems platings were made and *Bact. lachrymans* obtained in pure culture. At the same time several control inoculations were made on stems and petioles, using a subculture of the fimbriate, peritrichiate, soft-rot organism plated from one of the softened cucumbers above mentioned, but no rot occurred (four weeks). This organism, however, soft-rotted green cucumber fruits when inoculated by needle pricks.

Last of all, following the discovery of Traverso's paper, another set of inoculations was made on cucumber fruits. Six marketable green hothouse fruits were selected and inoculated with *Bact. lachrymans*. At the end of 10 days in culture dishes at temperatures varying from 24° to 30° C. all showed local gumming and infection about the needle wounds, but none of them developed any soft-rot (Pl. XLVI, fig. 3).

HISTOLOGY OF DISEASED LEAVES

Pieces of a leaf that showed spotting were fixed on the second day, embedded, sectioned, and stained. Stomatal infections were very numerous (Pl. XLVII, fig. 1). The bacteria gorged the opening of the stoma in some cases, as well as the cavity beneath it. Even at this early date the bacteria had spread in great numbers for some distance from the stoma, crowding apart or crushing the cells of the parenchyma and causing a slight swelling on the leaf (Pl. XLVII, fig. 2).

MORPHOLOGY AND PHYSIOLOGY OF BACTERIUM LACHRYMANS

MORPHOLOGICAL CHARACTERS

As it occurs in the plant and also on media the organism causing the disease is a short rod with rounded ends, single or in pairs (Pl. XLVIII, fig. 2 and 3), 0.8μ wide by 1 to 2μ long. On culture media it occurs singly or in pairs with a very decided constriction, and occasionally (in salted bouillons) in chains of as many as 12 or more individuals (Pl. XLVIII, fig. 1). No spores have been seen. Capsules are formed on agar (Pl. XLVIII, fig. 2), and in milk (Ribbert's stain). It is motile by means of 1 to 5 polar flagella (Pl. XLVIII, fig. 3). It is Gram-negative and is not acid-fast.

EFFECT OF DESICCATION

When drops from 24-hour peptone bouillon were placed on sterile covers in sterile Petri dishes and kept in the dark at room temperature, the organism was not killed by 21 days' drying, but it gave no growth when covers were dropped into suitable bouillon after 6 weeks' drying.

TEMPERATURE RELATIONS

The best growth was obtained at 25° to 27° C. There was no growth at 36° , though bouillon was weakly clouded at 35° C. Slow growth occurred at 1° in bouillon cultures (two weeks' time).

SENSITIVENESS TO SUNLIGHT

Agar plates, thin-sown, from an 8-day bouillon culture were exposed, bottom up on ice, to sunlight in June for 5, 10, and 15 minutes, one-half of each plate being protected from the light by several thicknesses of black paper. After five days' incubation numerous colonies appeared, and no difference was observed between the insulated and covered side on any of the six plates (but the colonies were not counted). Another test was made in September, 1915, with the following results:

The fluid used for inoculation consisted of one 3-mm. loop from a 24-hour bouillon culture into 10 c. c. of bouillon. Five plates were inoculated, each with one 2-mm. loop from this suspension. Five other plates were inoculated, each with one needle from this suspension. One plate from each lot was then half covered and exposed bottom up on ice for 5, 15, 30, 45, and 60 minutes, respectively. Result: All were killed by 45 and 60 minutes' exposure; three-fourths were killed by 30 minutes' exposure; one-third were killed by 15 minutes' exposure; and one-fourth were killed by 5 minutes' exposure.

When these results were obtained with the 24-hour bouillon, the experiment with the 8-day bouillon was repeated. Four agar plates were poured, one-half of each being exposed bottom up on ice, two for 15 minutes and two for 30 minutes, the sky being clear and the sun bright (October 12).

There was a marked reduction of colonies on the plates exposed for 15 minutes (estimated, 70 per cent), and almost complete absence of colonies on those exposed for 30 minutes (estimated, 95 per cent destroyed). The contradictory earlier result must therefore be attributed to a feebly actinic condition of the sky not visible to the naked eye.

SENSITIVENESS TO FREEZING

The organism is quite sensitive to freezing. A transfer was made to beef bouillon from a 5-day-old bouillon culture, shaken well and allowed to stand for five minutes. Plates were then poured with measured loops from this culture. The tube was then buried in salt and pounded ice, frozen solid and kept frozen for 15 minutes, after which it was thawed in cool water (five minutes required), shaken thoroughly, and used for a second set of plates, the loops being measured exactly as before. Two days after pouring the colonies were counted. There were one-ninth as many colonies after freezing as before freezing (Pl. XLVII, fig. 3). A longer incubation (five days) did not increase the number of colonies on the plates.

Thinking that five minutes might not have been long enough to obtain a uniform diffusion of the bacteria in the fluid, the experiment was repeated, allowing the tube to stand an hour with shaking before the plates were poured. The result was practically the same, nine-tenths of the bacteria being destroyed by the short freezing, the count being made on the fifth day.

CULTURAL CHARACTERS

AGAR-POURED PLATES.—On +15 peptone-beef agar at 23° C. surface colonies 2 days old are 1.5 to 2 mm. in diameter, round, smooth, shining, slightly convex, finely granular (under the compound microscope), with an opaque white center and a thin, transparent, entire margin. When 3 to 4 days old at 23° C. the largest measure 4 to 7 mm. in diameter and the white opaque center spreads in radiating lines into the thin margin (Pl. XLIX, fig. 1). At higher temperatures (27° to 30° C.) they reach this size in two to three days. Buried colonies are lenticular. Later (when 4 to 5 days old) the surface colonies lose their dense white center and dry down very thin and transparent and then show little or no trace of the radiating lines.

AGAR STABS.—Stabs in +15 peptone-beef agar when 2 days old at 23° C. show a raised, smooth, shining, white, transparent, surface growth 8 mm. in diameter. Growth is visible only along the upper one-third of the stab. This is granular, not villous.

Old cultures have a thin white growth completely covering the surface, and the agar is then frequently pale green, fluorescent.

AGAR SLANTS.—On slant agar, stroke cultures make a moderate, thin, white, transparent, smooth, shining growth, denser in the center. There is considerable white sediment in the V.

GELATIN PLATES.—Surface colonies on gelatin plates show a peculiar margin, best seen under low magnifications, with oblique light (Pl. XLIX, fig. 2). Liquefaction is slow (18° to 20° C.), and when the layer of gelatin is thin (10 c. c. to a plate) does not take place, as the medium soon becomes too dry for growth. On plates containing 20 c. c. of gelatin liquefaction began on the twelfth day and on the sixteenth day was complete, the colonies floating intact in the liquid gelatin.

GELATIN STABS.—At 15° to 18° C. in +10 peptone gelatin the surface growth after seven days is about 6 mm. in diameter, with a pit of liquefaction 2 mm. wide and 2 mm. deep. Stab growth is granular, not villous, fading out downward. As liquefaction progresses the upper part becomes stratiform, the lower part bluntly funnel-form (Pl. XLIX, fig. 3). Liquefaction progresses rather slowly but is complete within three to four weeks at the specified temperatures.

BEEF BOUILLON.—In +15 peptone-beef bouillon uniform clouding occurs within 24 hours. This clouding is weak to moderate, never strong. On the second day a membranous pellicle is formed, which fragments and falls readily on shaking. It is made up of a homogeneous mass of bacteria—i. e., free from pseudozoogloæ but containing a few short chains (10 or 12 individuals). Old cultures (4 to 6 weeks old) are often decidedly green fluorescent. The white precipitate breaks up readily on shaking and contains many small crystals.

POTATO CYLINDERS.—When inoculated from agar cultures growth on steamed potato cylinders in two days is moderate, spreading, creamy white, shining, and slimy. The part of the potato out of the water becomes slightly browned. Growth on potato soon ceases. After 10 days the color of the potato is completely changed, becoming a pale brownish hue, and the growth takes on a similar color (very pale brownish). Tested with alcohol iodine for starch, such cultures give a heavy dark-purple reaction, showing that there has been only a partial digestion of the starch (formation of amyloextrin). The cylinders are not softened.

MILK.—Inoculated milk clears slowly and without coagulation. Clearing begins within a week, and after two weeks tubes of it are translucent so that the outlines of a pencil back of the milk may be seen through it clearly. Cultures 1 month old are still clear but are then tawny olive,¹ with a darker rim where the milk has dried down.

LITMUS MILK.—Lavender-colored litmus milk begins to blue from the top downward on the second day and is completely blued by the third day, without a sign of coagulation or clearing. A decided creamy-white pellicle is formed.

After 10 days clearing begins and is complete in 20 days. Later the blue color bleaches out (reduction phenomena), beginning at the bottom, leaving the whole fluid a clear (translucent) brown. At no time is there any reddening of the litmus or any coagulation of the milk; nor are any crystals formed in it.

FERMENTATION TUBES.—The tests in fermentation tubes were made in water containing 2 per cent of Witte's peptone, to which was added 2 per cent of the carbon compound to be tested—namely, saccharose, dextrose, lactose, maltose, glycerin, and mannit. Clouding occurred in the open end of each on the second day, heaviest in the tubes containing saccharose and dextrose, but the closed end in every case remained clear, with a distinct line across the inner part of the U. When 5 days old they were tested with neutral litmus paper. Saccharose and dextrose gave a decidedly acid reaction, while all the others were neutral. When 20 days old the saccharose and dextrose were still acid and the others weakly alkaline. No gas was formed and no growth occurred in the closed end of any.

No gas was formed in fermentation tubes containing sterile milk; nor was there any separation of the curd. The milk in the open end cleared gradually, while that in the closed end remained unchanged. The litmus reaction was alkaline in the open end.

Nitrate bouillon in fermentation tubes gave a good clouding in the open end, none in the closed end, no gas, and no nitrate reduction. A decided alkaline reaction was obtained with neutral litmus paper.

TOLERATION OF SODIUM CHLORID.—Neutral peptone-beef bouillons containing 2, 5, 6, and 7 per cent of chemically pure sodium chlorid, respectively, were inoculated from young bouillon cultures. Growth was retarded by 2 per cent of sodium chlorid

¹ Ridgway, Robert. A nomenclature of colors . . . 129 p., 17 pl. (partly col.). Boston, 1886.

and inhibited by all the other strengths. The experiment was repeated using 2, 3, and 4 per cent of sodium chlorid. Again, the 2 per cent retarded growth (clouding on the fourth day). Checks clouded after 24 hours. Growth appeared in the 3 per cent after 12 days, but there was no growth in the 4 per cent even at the end of four weeks. In both 2 per cent and 3 per cent the growth was scanty and flocculent, composed largely of chains (Pl. XLVIII, fig. 1), especially in the 3 per cent solution.

TOLERATION OF ACIDS.—Neutral bouillon containing 0.1, 0.2, and 0.3 per cent, respectively, of malic acid, tartaric acid, and citric acid was used. After three days the 0.1 per cent cultures of all three acids were well clouded; the 0.2 per cent malic and tartaric acids were all moderately clouded, while the 0.2 per cent citric acid showed no growth. None of the 0.3 per cent cultures were clouded. After three weeks the 0.2 per cent citric acid was well clouded, but in no case did the 0.3 per cent cultures show any growth. The cultures were watched for five weeks.

TOLERATION OF ALKALI.—The organism is quite sensitive to alkali. Peptonized beef bouillons titrating, according to Fuller's scale, +25, +20, +10, +5, 0, -5, -20, and -30, were inoculated from a 4-day bouillon culture, using a carefully measured 3-mm. loop for each tube. After 24 hours all showed growth except the -20 and -30. Heaviest growth occurred in the +25, weakest growth in the -5, which was flocculent instead of clouded. Five days later the same relative growth was evident throughout the series, but the -5 had become clouded and the -20 weakly flocculent. The -30 remained clear. After two weeks there was moderate growth in the -20, but none in the -30. The alkali used was sodium hydrate.

USCHINSKY'S SOLUTION.—In Uschinsky's solution growth is heavy, with a heavy membranous pellicle which falls readily as a whole. Greening of the media begins at the top on the second or third day and proceeds rapidly downward until the whole is a decided pale apple green. The medium does not become viscid.

FERMI'S SOLUTION.—At the end of 10 days a fine green fluorescence like that in Uschinsky's solution is visible. No fluorescence appeared in tubes of Cohn's solution inoculated on the same date for comparison.

COHN'S SOLUTION.—There is good clouding, heaviest near the top, but without a pellicle. Numerous floating crystals occur and the white precipitate is dotted with crystals. No greening occurs.

SUGAR AGARS.—No yellowing occurred on any of the sugar agars used. Cultures were made on beef-peptone agars containing, respectively, 2 per cent of saccharose, maltose, and dextrose, and in sugar agar without beef—i. e., containing only peptone and saccharose. The cultures were watched for eight weeks, during which time they remained white.

DOLT'S SYNTHETIC AGAR.¹—Growth is abundant, covering the surface on the third day with a thin pink layer. Reddening of the dark agar begins on the second or third day; and after 10 days the color is changed throughout, although the lower half has not lost completely its purplish hue.

BOUILLON OVER CHLOROFORM.—Growth is not retarded in unshaken tubes of peptone-beef bouillon to which 5 c. c. of chloroform have been added.

REDUCTION OF NITRATES.—Nitrates are not reduced. Five-day-old cultures in nitrate bouillon were tested by the addition to each of 1 c. c. of boiled starch water, 1 c. c. of potassium-iodid water, and 10 drops of sulphuric acid. There was no color reaction.

INDOL.—There is a weak indol production in 2 per cent peptone water and in peptonized Uschinsky's solution. Tests were made at the end of the fifth and tenth days by the addition of 1 c. c. of the standard sodium-nitrite solution and 10 drops of the sulphuric-acid water to each tube. No reaction appeared until the cultures were heated to 70° C., when a feeble but decided pink color appeared. The checks gave no pink reaction. A better reaction was obtained in peptone water containing 0.5 per cent of sodium chlorid (Dunham's solution)—about one-third that of *Bacillus coli*.

¹ Contains litmus, glycerin, milk sugar, and dibasic ammonium phosphate.

HYDROGEN SULPHID.—Strips of filter paper soaked in strong lead-acetate solution and dried were suspended over cultures in peptone-beef bouillon, milk, steamed potato, carrot, and turnip. No browning of the paper occurred within six weeks.

METHYLENE BLUE IN MILK.—Methylene blue is rapidly reduced. Cultures were made in milk containing 4 per cent of a 1 per cent solution of methylene blue. Bleaching begins on the second day and is complete or nearly so in six days, except for a pale-blue surface layer 2 to 4 mm. deep and a deep-blue rim and pellicle. This pellicle, when examined under the microscope, is seen to be composed of masses of bacteria that have taken up the stain. When shaken repeatedly, these bleached cultures regain their blue color.¹

BLOOD SERUM.—Stroke cultures on Loeffler's blood serum give a moderate, white, shining filiform growth 3 mm. wide. There is no liquefaction even after eight weeks and no color change in the substratum.

AEROBISM.—The organism appears to be strictly aerobic. It does not grow in the closed end of fermentation tubes with any carbon food tested. In agar stab cultures no growth occurs in the lower end of the stab. Cultures were also made by shaking an inoculated tube of melted agar, but no growth occurred more than 3 mm. below the surface. Stabs were made in agar, then 10 c. c. of melted agar poured on top. No growth occurred in the stab or at the junction point, but there was good growth on the exposed surface of the added agar.

LITMUS AGAR WITH SUGARS.—On litmus-lactose-agar stroke cultures there is moderate growth and no color change.

Stroke cultures on litmus-maltose agar give heavy growth, but do not alter the color.

On litmus-saccharose agar growth is heavy and the medium reddens, beginning at the thin upper end. The reddening begins on the second or third day and is complete on the fifteenth day.

Following the chart of the Society of American Bacteriologists, the group number is 211.2321*23.

EFFECT OF COPPER SULPHATE ON THE ORGANISM

Bouillon cultures 24 hours old were exposed to the action of chemically pure copper sulphate in the following manner. A dilution of copper sulphate (1 to 1,000) was made in a large Jena flask and allowed to stand overnight. After shaking thoroughly, further dilution was made again (in liter quantities) to 1 to 100,000 and 1 to 500,000. After these had been well shaken and had stood for an hour 10 c. c. of each were put into sterile test tubes and a loop of a well-clouded suspension from a 24-hour-old agar culture was added. Plates were poured after 5, 10, 20, and 30 minutes from each tube, using carefully measured loops. Checks were made by pouring plates with the same measured loops from a similar dilution in sterile water.

The plates were incubated at room temperature (27° to 30° C.). A colony count was made on the second day. Exposure to the 1 to 500,000 dilution gave no observed reduction of colonies, but the 1 to 100,000 destroyed nine-tenths of the organisms. The experiment was repeated with a strength of 1 to 50,000 of copper sulphate. All were killed at this exposure, while the check gave numerous colonies.

¹ The blue pigment is also absorbed by the bacteria from peptone water containing methylene blue.

*Nonchromogenic on most media, but green fluorescent in Uschinsky's solution, Fermi's solution, and old peptone-beef bouillon.

Some weeks later the experiment with copper sulphate was repeated. To liter quantities of distilled water in Jena flasks, chemically pure copper sulphate was added so as to obtain the following dilutions: 1 to 50,000; 1 to 100,000; and 1 to 500,000. Some hours after full solution, 10 c. c. of each dilution were pipetted into sterile test tubes and to each was added a 3-mm. loop from a heavily clouded water suspension made from a 24-hour agar slant culture. From each of these tubes three plates were then poured at the end of 5 minutes, and again three more at the end of 10 minutes. As a check, a 3-mm. loop of the cloudy bacterial suspension was added to 10 c. c. of distilled water and from this tube three plates were also poured. The agar for the first set of poured plates was seeded with a 3-mm. loop from the dilution tube, that for the second set with a 2-mm. loop, and that for the third set with a needle dipped one-half inch into the fluid. The results in colonies are given in Table II, the counts being made on the sixth day.

TABLE II.—Effect of copper sulphate on *Bacterium lachrymans*

Dilution used.	Checks.	Number of colonies of <i>Bacterium lachrymans</i> developing in—					
		1 to 50,000 copper sulphate.		1 to 100,000 copper sulphate.		1 to 500,000 copper sulphate.	
		5-minute exposure.	10-minute exposure.	5-minute exposure.	10-minute exposure.	5-minute exposure.	10-minute exposure.
Plate 1 (3-mm. loop)...	3, 844	78	45	118	55	3, 412	1, 756
Plate 2 (2-mm. loop)...	2, 296	27	16	29	44	2, 400	916
Plate 3 (needle).....	22	0	0	0	0	12	5

SUMMARY

(1) The angular leaf-spot of cucumbers is a widespread disease occurring in many of the Eastern and Middle Western States.

(2) It is characterized by angular brown spots which tear or drop out when dry, giving to the leaves a ragged appearance. In the early stages a bacterial exudate collects in drops on the lower surface during the night and dries whitish.

(3) Young stems and petioles may become soft-rotted or cracked open.

(4) A virulent outbreak often materially reduces the crop by destroying the needed active leaf surface.

(5) The spot is caused by *Bacterium lachrymans*, n. sp., which enters through stomata, no wounds being necessary. This organism is quite different from the one described by Burger¹ in his papers on cucumber rot. No direct connection has been found between the leaf-spot and the soft-rots of the fruit.

(6) Considering the results obtained in the laboratory with copper sulphate, it would seem that Bordeaux mixture properly applied is the remedy for this disease. Thorough field tests with it should at least be undertaken where the disease is troublesome.

¹ Burger, O. F. Op. cit.

PLATE XLIII

Fig. 1.—Cucumber leaf eight days after inoculation with *Bacterium lachrymans*. The bacterial exudate has now dried down into white crusts.

Fig. 2.—Cucumber leaf 12 days after spraying with *Bact. lachrymans*. Diseased tissue shriveled and spots falling out.

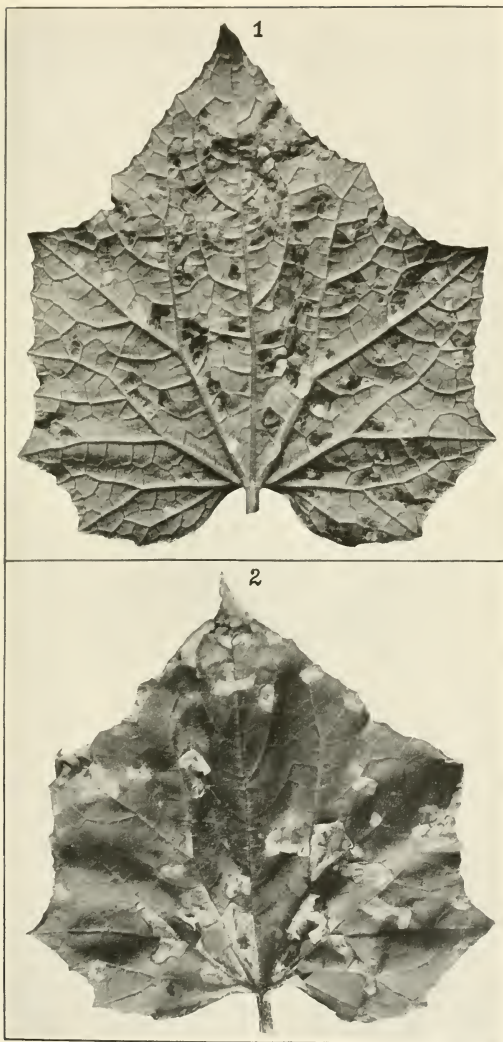




PLATE XLIV

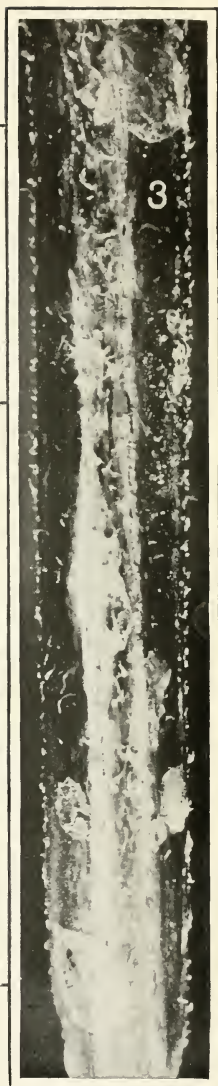
Cucumber stem diseased by *Bacterium lachrymans*. The white bacterial exudate may be seen at X, X. Photographed 14 days after spraying.

PLATE XLV

Fig. 1.—Fragment of a cucumber leaf showing angular leaf-spots due to pure-culture inoculation with *Bacterium lachrymans*. Time, six days. The glistening tearlike exudate can be seen in a number of places. $\times 2$.

Fig. 2.—Cucumber plant 18 days after spraying with *Bact. lachrymans*. Upper part of stem softened and shriveled. Lower part as at X with canker-like cracks which show bacterial exudate.

Fig. 3.—Stem at X in figure 2 enlarged to show bacterial lesions.



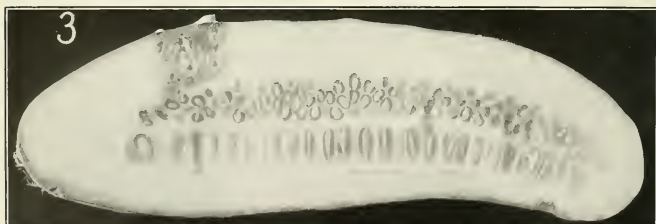
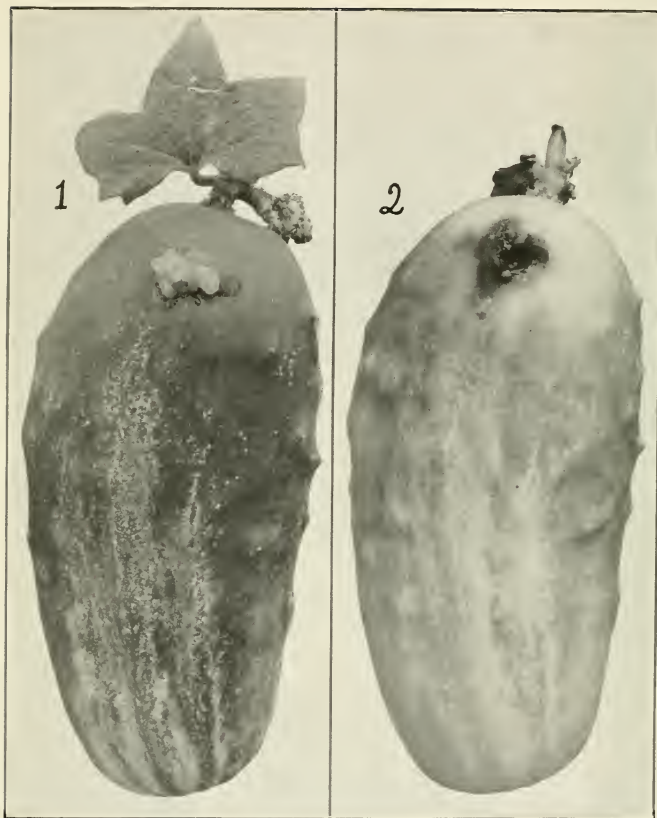


PLATE XLVI

Fig. 1.—Green cucumber fruit photographed six days after inoculation with *Bacterium lachrymans*. There is an exudate at the point inoculated (upper part of fruit), while the remainder of the fruit is sound.

Fig. 2.—Same fruit as shown in figure 1, but at the end of 12 days. The fruit, which was slowly ripening, was still sound both externally and within, except at the point inoculated.

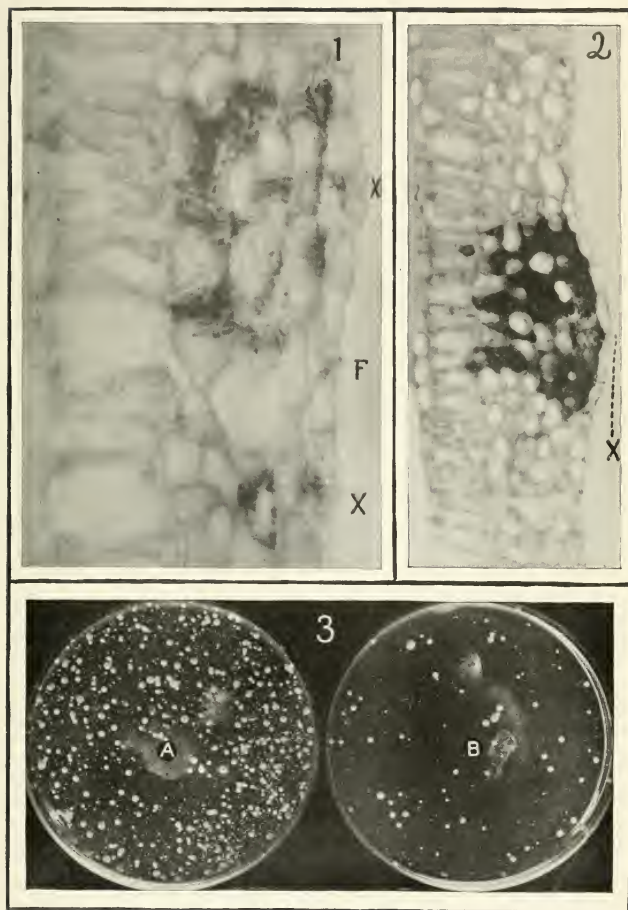
Fig. 3.—Section of green cucumber fruit 10 days after inoculation with *Bact. lachrymans* (6 days at 24° and 4 days at 30° C.). Not from the same series as figures 1 and 2. Tissue decayed only in the vicinity of the needle wounds.

PLATE XLVII

Fig. 1.—Cross section of a cucumber leaf, showing two stomatal infections (X, X). At *F* there is a third stoma whose chamber is free from bacteria. Stained with carbol fuchsin. $\times 1,000$, nearly.

Fig. 2.—Cross section of cucumber leaf showing a dense bacterial infection due to *Bacterium lachrymans*. Stoma at X. Moderate magnification. Carbol-fuchsin stain. Tissues pushed out.

Fig. 3.—*A*, Agar-poured plate from bouillon dilution of *Bact. lachrymans*; *B*, agar-poured plate made from same quantity of same bouillon as *A*, but after freezing 15 minutes.



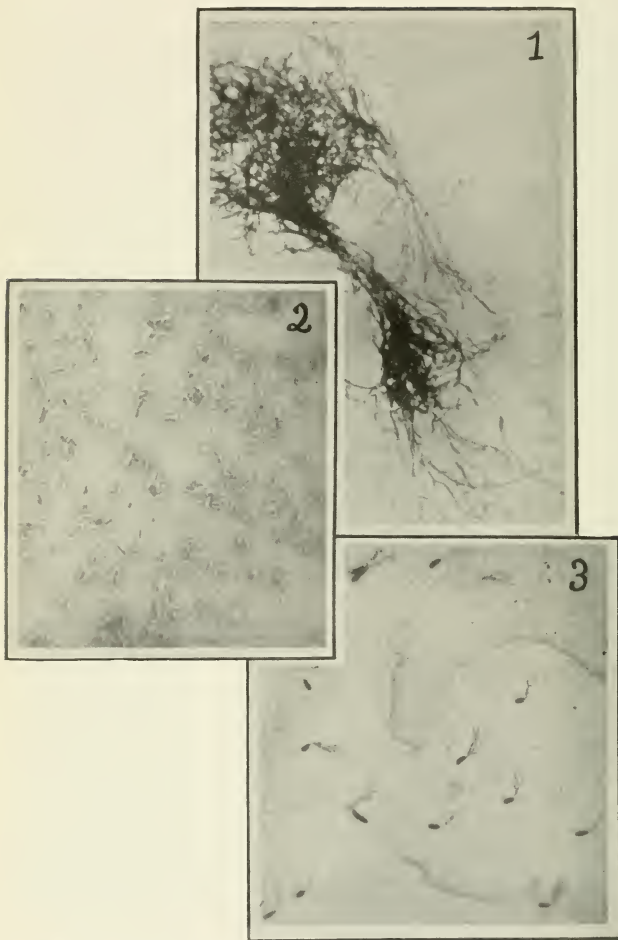


PLATE XLVIII

Fig. 1.—Chains of *Bacterium lachrymans* from 14-day-old culture in salted bouillon. Stained with carbol fuchsin. $\times 1,000$.

Fig. 2.—Capsules of *Bact. lachrymans* from young agar culture. Ribbert's capsule stain. $\times 1,000$.

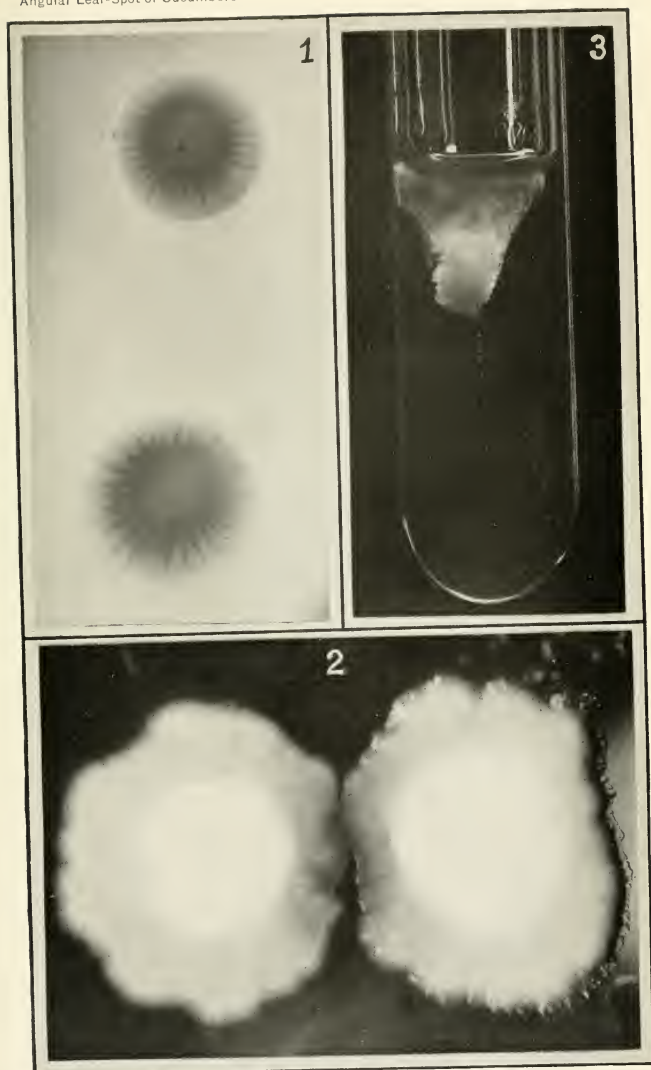
Fig. 3.—Flagella of *Bact. lachrymans* from 24-hour-old agar slant. Stained by Van Ermengem's silver-nitrate method. $\times 1,000$.

PLATE XLIX

Fig. 1.—Young surface colonies of *Bacterium lachrymans* on agar poured plate, showing opaque center and lines radiating into the thinner margin. $\times 14$.

Fig. 2.—Surface colonies of *Bact. lachrymans* on gelatin poured plate. Photographed to show characteristic margin. $\times 14$.

Fig. 3.—Gelatin stab culture of *Bact. lachrymans*, kept at 20° C. and photographed at the end of 12 days. Liquefaction confined to the top, but a discrete growth along the line of the stab nearly to the bottom of the tube.



ACTIVITY OF SOIL PROTOZOA¹

By GEORGE P. KOCH,

*Research Fellow, the New Jersey State College for the Benefit of
Agriculture and Mechanic Arts*

INTRODUCTION

The belief that soil protozoa are destructive to bacteria and, hence, are influencing factors in soil fertility is encouraging the more extended study of these organisms. It was shown elsewhere (5)² that the soil contains many cysts of protozoa which become active under favorable conditions. To serve as limiting factors in the soil, protozoa must be present in the active condition, for it is only as such that they can destroy bacteria and other micro-organisms; thus, the question at once presents itself, Are the protozoa active in the soil?

In 1909 Wolff (13) recorded investigations with soil protozoa undertaken for the purpose of ascertaining whether these organisms lead an active life in the soil and of discovering the factors which influence their activity. As to the presence of protozoa in the soil, Goodey (2), in 1911, concluded that they were not active in normal soils. A few years later, however, he (4) found that ciliated protozoa are in the encysted condition and concluded that the amebæ and flagellates were the limiting factors in the soil. Martin and Lewin (7) upon examining cucumber-sick soils found several different kinds of protozoa. The amebæ were probably the dominant type, and the flagellates were comparatively few. In 1911 Russell and Golding (9) noted that species of *Vorticella*, *Putrina*, *Euglena*, and other types present in ordinary soils were also found in sewage-sick soils. These organisms were more active in the sewage-sick soil than in ordinary field soil. In 1913 Russell and Petherbridge (11), in studying "sickness" in cucumber soil, found it to be full of organisms like myxomycetes, active amebæ, eelworms, and other lower animal forms.

Sherman (12, p. 630), who studied the presence of protozoa in several types of soil, summarizes his observations as follows:

Certain forms of the soil protozoa are active under normal, and even sub-normal, conditions of moisture. The active protozoan inhabitants of most soils are probably restricted to flagellates. *Colpoda cucullus* is probably active whenever the moisture content is much above normal but does not appear to be so ordinarily.

¹Contribution from the Laboratories of Protozoology, Soil Bacteriology, and Soil Chemistry of the New Jersey Agricultural College and Experiment Station.

²Reference is made by number to "Literature cited," p. 488.

As to the activity of soil protozoa, Cunningham (1, p. 56) states:

To the question as to whether the protozoa lead an active life in the soil, it has been shown that the action of heat combined with the dilution method does not give a definite answer. That question, however, is answered in the affirmative by the results of experiments which will now be discussed.

Martin and Lewin (8, p. 117) likewise in a recent article concluded that "it seems probable from the work that we have done up to the present that there are always some free living protozoa present in a trophic state in even relatively dry, poor soils."

In this study it is the purpose of the writer—

- (1) To develop a method for studying protozoan activity in the soil.
- (2) To ascertain whether the protozoa lead an active life in soils of different moisture content when the temperature is constant and when it is variable.
- (3) To study the effect of moisture on the activity of the protozoa in the soil under constant and variable temperatures.
- (4) To study the length of the period of excystment of soil protozoa.

METHOD FOR STUDYING PROTOZOAN ACTIVITY IN THE SOIL

In studying the activity of protozoa in the soil the first difficulty which is encountered is the lack of a suitable method by which the investigator can determine with certainty the extent to which these organisms are active in the soil. Several methods are recorded that have been used with more or less success. In 1911 Goodey (2) passed an electric current through the medium and found that the living protozoa traveled with the current to the cathode. The separation of active forms by centrifugation was attempted by Russell and Golding (10) in 1912. In 1913 Martin (6) discussed a simple method based on the mixing of a small quantity of soil with picric acid and then noting the organisms (bacteria, protozoa, and diatoms) which rose to the surface, when this mixture was placed in a wide dish and the soil stirred. Cunningham (1) employed the dilution method for examining and counting the protozoa in the soil. Martin and Lewin (8) discuss several methods which they have employed with more or less success. For the detection of living amebæ, an air-blast method which they have devised has proved to be the most successful.

It was suggested by Martin and Lewin (8, p. 110) that—

Any method which depends upon the addition of water to the soil must admit of very rapid execution, otherwise there is danger of protective cysts present in the soil opening, and thus giving a false impression as to the constitution of the active fauna. This danger is probably a very real one in the case of small flagellates, and especially the resting forms of some green algæ, in the case of which a few minutes' immersion in water may make the difference between a resting and an active form.

In order to determine the presence of motile protozoa in the soil, the writer has found the direct method of examining the soil to which a little water has been added the most satisfactory.

Several drops of sterile tap water (15 pounds' pressure for 15 minutes) are placed on a clean slide; then by means of a stirring rod a small portion of soil is stirred in this water and spread out in a thin film, so that the observer can readily see between the soil particles. Examinations are then quickly made under the low power (16 mm. lens) of the microscope.¹ As soon as the soil touches the water, the time is recorded and the examination is continued for a period of not more than two minutes, in this way reducing the possibility of error which the observer might make on account of the rapid excystment of the protozoa, as was suggested above.

PROTOZOAN ACTIVITY IN SOILS OF DIFFERENT MOISTURE CONTENT AND UNDER CONSTANT AND VARIABLE TEMPERATURES

GREENHOUSE SOILS

The conclusions of other investigators as to the presence of protozoa in the active state in normal soils led the writer to examine greenhouse and field soils for the purpose of finding out, if possible, to what extent the protozoa were present in the active state in the different soils.

Twenty greenhouse soils of different composition and texture were examined, each for half an hour, a new sample being placed on the slide every two minutes. These samples were all taken at a depth of 1 inch from the surface. The examinations were all made in the greenhouse. The results are given in Table I.

From Table I it is seen that protozoa can and do exist in the active state in greenhouse soils. Their presence, however, is very limited, as they were found in but 6 out of the 20 soils examined. All the soils in which the protozoa were found were of open structure and their moisture content was much above their optimum. A compact shale soil with added manure and high moisture content did not show any living protozoa. Soils with a large proportion of organic matter and with a relatively low percentage of moisture did not seem to encourage the presence of active protozoa. From the data presented it would seem that the moisture content is the primary limiting factor, while the texture and content of organic matter are secondary.

¹ In studies previously recorded (1), all the examinations were made under the low power of the microscope, as it was not possible to distinguish between motile bacteria and what might be called "protozoa." In the studies referred to, no difficulty was encountered in seeing protozoa which were as small as species of *Bodos* or *Monos*; hence, the data collected in this study are based on the examinations made under the low power of the microscope.

TABLE I.—Extent of protozoan activity in greenhouse soils

Lab- ora- tory No.	Kind of soil.	Fertilizer treatment.	Termpera- ture.	Moisture content.	Presence of protozoa. ^a
			°C.	Per cent.	
1	Clay loam.....	20 per cent of compost + minerals.	20. 8	26. 65	
2	Shale.....	20 per cent of compost.....	20. 9	34. 30	
3	Clay loam.....	20 per cent of compost; 20 per cent of sand.	21. 0	26. 66	S.C.† A.†
4	Sandy.....	20 per cent of compost.....	21. 0	26. 84	
5	Clay loam.....	40 per cent of compost.....	24. 0	36. 27	
6	Shale.....	20 per cent of compost; 30 per cent of sand.	22. 7	25. 17	
7	Sandy loam....	No mixture.....	21. 6	22. 59	S.C.†
8	Clay loam.....	20 per cent of compost; 20 per cent of sand.	21. 1	27. 57	
9do.....	40 per cent of compost.....	21. 1	35. 75	
10	Sandy.....	20 per cent of compost.....	20. 8	26. 59	S.C.†
11do.....	40 per cent of compost.....	23. 0	35. 35	S.C.† F.†
12	Sandy loam....do.....	22. 7	31. 28	
13do.....	20 per cent of compost.....	22. 5	29. 10	S.C.†
14	Clay loam.....	20 per cent of compost + minerals.	24. 6	27. 90	S.C.†
15	Shale.....	20 per cent of compost + 10 per cent of sand.	21. 0	31. 75	
16	Clay loam.....	No mixture.....	19. 0	26. 21	
17do.....	20 per cent of compost + minerals.	20. 3	31. 07	
18	Sandy loam....	20 per cent of compost.....	24. 0	25. 81	
19	Clay loam.....	20 per cent of compost; 20 per cent of sand.	24. 6	25. 09	
20do.....	No mixture.....	18. 0	26. 60	

^a S.C.=small ciliates; L.C.=large ciliates; F.=flagellates; A.=amebæ; †=few; ††=several; †††=many.

FIELD SOILS

The extent of protozoan activity in field soils was studied in the same manner as the greenhouse soils. Samples of 14 field soils of different texture and tillage treatment were collected at a depth of 3 inches from the surface and brought to the laboratory in flasks. The temperature was in all cases noted. These were examined at once, each for half an hour, a new sample being placed on the slide every two minutes, as in the case of greenhouse soils. The moisture content was likewise determined. The soils were sampled and examined under normal conditions, again two days after a fall of 1.69 inches of rain, and a third time five days after 1.69 inches of rainfall. The second sampling was made at that period, since it allowed the organisms sufficient time to excyst, if possible, when the moisture content of the soil was increased. Likewise, the third examination was made five days after the heavy rainfall, for if the protozoa excysted and were washed to a lower level in the soil, this lapse of time allowed them to return to their normal level in the soil. Each soil was subjected to a half-hour's examination at every

sampling. In order to ascertain whether the soils contained cysts of protozoa which would become active when conditions became favorable after they had been examined, the soils collected at the third sampling were water-logged with sterile tap water and allowed to stand in the laboratory for 40 hours, when they were examined for motile protozoa. (See Table II.)

TABLE II.—Extent of protozoan activity in field soils under different conditions of moisture^a

Laboratory No.	Kind of soil.	Soil treatment.	Normal moisture content.	Moisture content two days after heavy rain.	Moisture content five days after heavy rain.	Presence of active protozoa when soil samples were water-logged. ^b
			<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	
1	Shale.....	Bare.....	25.07	22.01	21.73	S.C.††† F.†
2	Sandy loam.....	Orchard...	13.73	18.66	14.18	S.C.†† F.††
3	Gravelly sandy loam.	Garden....	9.62	12.40	8.60	S.C.† F.†
4	Clay loam.....	Orchard...	15.14	19.18	12.72	S.C.† F.†
5	Gravelly clay...	Meadow...	15.67	20.10	15.21	S.C.††† L.C.† F.†
6	Clay loam.....	do.....	19.65	17.30	14.88	S.C.† L.C.† F.†
7	Silt loam.....	Wheat....	11.22	15.24	9.38	S.C.†† F.†
8	do.....	Weeds....	13.42	16.08	14.62	S.C.†† L.C.† F.†
9	Sandy.....	Corn.....	11.34	14.88	11.25	S.C.†† F.††
10	Gravelly silt loam.	Fallow....	10.93	14.22	10.58	S.C.†† F.†
11	Shale.....	Bare.....	19.88	23.36	20.27	S.C.††† L.C.† F.†
12	Gravelly silt loam.	Wheat....	9.60	15.51	8.97	S.C.† F.†
13	Silt loam.....	Corn.....	10.90	15.66	10.95	S.C.†† F.††
14	Sandy loam.....	Vetch and tomatoes.	6.74	12.18	8.52	Do.

^a Under normal conditions and two and five days after a heavy rain no active protozoa were observed.

^b S. C. = small ciliates; L. C. = large ciliates; F. = flagellates; A. = amebæ; † = few; †† = several; ††† = many.

The careful examination of the 14 soils in no case revealed any motile protozoa, indicating that under the normal and even somewhat abnormal conditions of moisture active protozoa did not seem to be present in the soils examined. Several samples of standing rain water were collected when the second and third samplings were made. Upon examination all of the samples of water showed the presence of many small ciliates and flagellates, which indicates that the protozoa are active in accumulated water. In all cases where the 14 soils were water-logged small ciliates and flagellates, and in some cases even large ciliates, were present in the active state. The data presented in Table II point to the fact that all ordinary soils contain cysts of protozoa, and in the 14 soils examined the active organisms were not observed until sufficient moisture was present. It would seem that if the protozoa did become active when the moisture content was higher than it was at the time of the first sampling after the heavy rain, they remained active but a very short period of time, as in no case were they

found in the living condition, while in soils of very open structure where little or no surplus water is available they would seldom, if ever, become active. This point requires further investigation.

The question at once arises, How are protozoan cysts transported to the different soils? This process is likely to be brought about by wind action, by flowing water, and by mechanical means in the case of cultivated soils. Likewise, if the protozoa do not exist in the active state in the soil, can they and do they multiply? Under certain abnormal conditions of moisture they will become active and remain active as long as there are sufficient moisture and food and the absence of toxic or decomposition products. During this period multiplication takes place. When the conditions become unfavorable, no doubt some die, while the greater number encyst until conditions again become favorable for them to become active.

EFFECT OF MOISTURE ON THE ACTIVITY OF PROTOZOA IN THE SOIL UNDER CONSTANT AND VARIABLE TEMPERATURES

Large samples of three soils which had previously been used by the writer (1) in his study of protozoa were collected. The first was a 20 per cent manure shale, greenhouse soil, the second, a clay loam orchard soil which had received no applications of manure for the last 20 years; and the third, a sandy loam field-plot soil that for a period of 20 years had been receiving annual applications of manure at the rate of 20 tons per acre. (Hereafter throughout this study the first soil will be designated as the "greenhouse soil," the second as the "orchard soil," and the third as the "field soil.") The soils were air-dried at laboratory temperature and then sieved through a 20-mesh sieve. The optimum moisture content of these soils was determined. Twenty 50-gm. portions of each soil were weighed into 4-ounce bottles. With each soil one series of five samples was left air-dried. To one series sufficient sterile tap water was added to make the moisture content half of the optimum. To another series enough water was added to increase the water content to the optimum. To a fourth series sterile tap water was added so that the resulting mixture would be equivalent to one and a half of the optimum. At one and one-half of the optimum the soils could take up all the moisture without any free water being present. The soils were well mixed with a stirring rod, so that the moisture content was homogeneous throughout. In order to prevent condensation on the sides of the bottles, they were left unplugged. The flasks containing four samples of each soil, representing four moisture contents, were incubated at 5° to 7° C., one series at 15° to 17°, one at 22° to 24°, one at 32° to 33°, and one at the outdoor temperature. The samples were weighed daily, and the slight amount of moisture lost by evaporation was replaced. Each sample of soil was then examined for active protozoa not fewer

than three times, a new sample being taken every two minutes; during the examinations the respective samples were kept at the different temperatures. Sterile tap water of the same temperature as that at which the respective soils were incubated was used in making the examinations. Each series of samples were kept screened from the light during the period of incubation. After examination the samples were again weighed to determine the quantity of soil used in examination. Daily examinations of each sample of each soil were made for a period of eight days. (See Table III.)

TABLE III.—*Presence of active protozoa in different soils, with varying amounts of moisture at different temperatures (constant and variable) for a period of eight days*

Lab- ora- tory No.	Kind of soil.	Mois- ture added to 50 gm. of soil.	Relative moisture.	Mois- ture content on the oven- dry basis.	Tem- pera- ture of incuba- tion, ^a	Presence of protozoa after inoculation (days).							
						1	2	3	4	5	6	7	8
1211	Green house soil.	Gm. 0	Air-dry.....	Per ct. 0.69	° C. 15 to 17
1212do.....	4.96	½ optimum....	9.64do.....
1213do.....	9.92	1 optimum.....	17.12do.....
1214do.....	14.95	1½ optimums....	23.54do.....
1311	Orchard soil.	0	Air-dry.....	.28do.....
1312do.....	4.47	½ optimum.....	8.46do.....
1313do.....	8.93	1 optimum.....	15.39do.....
1314do.....	13.41	1½ optimums....	21.37do.....
1411	Field soil.....	0	Air-dry.....	.14do.....
1412do.....	3.48	½ optimum.....	6.63do.....
1413do.....	6.95	1 optimum.....	12.32do.....
1414do.....	10.41	1½ optimums....	17.34do..... ^b S. C.†

^a The writer did not think it advisable to include the remainder of Table III representing samples incubated at 5° to 7°, 22° to 24°, 32° to 33°, and at the outdoor temperature, as in no case were any living protozoa found during the period of eight days.

^b S. C.†=few small ciliates.

Upon examining Table III it is seen that in but one sample of soil (the field soil which had an optimum and a half of moisture) were any active protozoa observed. It was noted that there was a little depression in the sample of soil and a little free available water was present, thus no doubt accounting for the presence of this organism on the third day of incubation, as on no other day and in no other soil were any motile protozoa seen.

In order to be certain that these soils contained cysts of protozoa and to collect some data as to the amount of moisture necessary for excystment and also to note the time of excystment of protozoa when conditions are favorable, to each series of the three different soil samples containing moisture to the amount of half optimum and optimum and a half sterile tap water was added to make the amount two optimums and two and one-half optimums, respectively. These samples were then incubated at the same temperatures as before, and daily examinations for a period of four days were made. (See Table IV.)

TABLE IV.—*Presence of active protozoa in different soils at different temperatures when the moisture conditions were favorable*

Lab- oratory No.	Kind of soil.	Mois- ture cont- ent.	Relative amount of moisture (in opti- mums).	Temperature of incubation.	Presence of active protozoa. ^a			
					8 to 12 hours after inocu- lation.	30 to 36 hours after inocu- lation.	Third day after inoculation.	Fourth day after inocu- lation.
		<i>Per ct.</i>		<i>°C.</i>				
1202	Greenhouse...	28.88	2	5 to 7.....				F.†
1204do.....	36.66	2½	5 to 7.....				S.C.† F.†
1302	Orchard.....	26.52	2	5 to 7.....				F.†
1304do.....	29.05	2½	5 to 7.....				F.†
1402	Field.....	21.83	2	5 to 7.....	S.C.†	S.C.†	S.C.	
1404do.....	25.86	2½	5 to 7.....	F.†	F.†	F.†	
1212	Greenhouse...	28.88	2	16 to 17.....			F.†	S.C.†
1214do.....	33.66	2½	16 to 17.....			S.C.†† F.††	S.C.†
1312	Orchard.....	26.52	2	16 to 17.....		S.C.†	S.C.† F.†	F.†
1314do.....	29.05	2½	16 to 17.....		F.†	S.C.† F.††	F.†
1412	Field.....	21.83	2	16 to 17.....		S.C.†	S.C.† F.†	S.C.† F.†
1414do.....	25.86	2½	16 to 17.....		F.†	F.††	F.†
1222	Greenhouse...	28.88	2	22 to 24.....			S.C.†† F.††	F.†
1224do.....	33.66	2½	22 to 24.....		S.C.† F.†	S.C.†† F.††	S.C.† F.††
1322	Orchard.....	26.52	2	22 to 24.....			S.C.†	F.††
1324do.....	29.05	2½	22 to 24.....		S.C.†	S.C.† F.†	F.††
1422	Field.....	21.83	2	22 to 24.....		S.C.†	S.C.†† F.††	S.C.†
1424do.....	25.86	2½	22 to 24.....		S.C.† F.†	S.C.†	S.C.†
1232	Greenhouse...	28.88	2	32 to 33.....			F.†	S.C.†
1234do.....	33.66	2½	32 to 33.....		S.C.† F.†	S.C.†	S.C.††
1332	Orchard.....	26.52	2	32 to 33.....		S.C.†	S.C.†† L.C.† F.†	F.†
1334do.....	29.05	2½	32 to 33.....	S.C.†	F.†	S.C.†† F.††	F.†
1432	Field.....	21.83	2	32 to 33.....	F.†	F.†	S.C.†† L.C.† F.†	S.C.† F.†
1434do.....	25.86	2½	32 to 33.....		S.C.† F.†	S.C.††	S.C.††
1242	Greenhouse...	28.88	2	O u t d o o r t e m p e r a - t u r e .			S.C.†	S.C.† L.C.†
1244do.....	33.66	2½do.....			S.C.†	F.†
1342	Orchard.....	26.52	2do.....		S.C.† F.†	S.C.†† F.†	F.†
1344do.....	29.05	2½do.....		S.C.†	S.C.† F.††	S.C.† F.††
1442	Field.....	21.83	2do.....		S.C.† F.†	S.C.† F.†	L.C.† F.†
1444do.....	25.86	2½do.....		F.†	S.C.† F.†	S.C.† F.†

^a S.C.=small ciliates, L.C.=large ciliates, F.=flagellates, A.=amebæ, †=few, ††=several, †††=many.

The data presented in Tables III and IV again point to the fact that the supply of sufficient moisture is the limiting factor which influences the presence of protozoa in the active state in the soil, while the temperature, the presence of organic matter, and the soil structure seem to be only secondary factors.

On examining Table IV it becomes apparent that the temperature influences the period of excystment, in that a higher temperature may encourage a more rapid excystment of a greater number of protozoa and that the physical character of the soil may be more or less influential in the movement of the organisms in the soil; yet if the moisture content is not high enough, the protozoa will not be present in the active state.

To find out whether protozoa were always present in the active state in water-logged soils, samples of six soils, three greenhouse and three field soils, which were kept in the laboratory for some time, were put into small bottles, water-logged, and the bottles plugged with rubber stoppers to prevent evaporation, and then allowed to stand in the labo-

ratory. Examinations were made from time to time for a period, and then the samples were placed outside in the open air where the temperature variation was great and examinations were again made. (See Table V.)

TABLE V.—*Presence of active protozoa in water-logged soils, under constant and variable temperatures*

Laboratory No.	Kind of soil.	Presence of protozoa when incubated at room temperature on— ^a		
		May 25.	June 4.	June 7.
1501	Greenhouse.....	S.C.† L.C.†.....	S.C.††† L.C.† F.†††...	S.C.† L.C.†
1502	...do.....	S.C.††† L.C.†† F.†††	S.C.††† F.††.....	S.C.††† F.††
1503	...do.....	A.†		
1504	Field.....	S.C.††† F.††.....	S.C.†† F.††.....	S.C.††† F.†
1505	...do.....	S.C.††† L.C.† F.††.....	S.C.†† F.††.....	S.C.†††† F.†††
1506	...do.....	S.C.††† F.†.....	S.C.†††.....	S.C.†††
		S.C.††† A.†.....	S.C.††† L.C.† F.†.....	S.C.†††
Laboratory No.	Kind of soil.	Presence of protozoa when incubated at outdoor temperature on— ^a		
		June 8.	June 16.	June 23.
1501	Greenhouse.....	S.C.† L.C.†.....	S.C.† L.C.† F.†.....	S.C.† F.†
1502	...do.....	S.C.††† F.†.....	S.C.††† L.C.††.....	S.C.† F.†
1503	...do.....	S.C.†††	S.C.†††	S.C.†
1504	Field.....	S.C.††† L.C.† F.†.....	S.C.††† L.C.††.....	S.C.† F.†
1505	...do.....	S.C.†††	S.C.†††	S.C.† F.†
1506	...do.....	S.C.†† L.C.†.....	S.C.††† F.†.....	S.C.†† F.†

^a S. C. = small ciliates; L. C. = large ciliates; F. = flagellates; A. = amebæ; † = few; †† = several; ††† = many.

The data given in Table V indicate that living protozoa were always present in all of the water-logged soils during incubation at outside temperature as well as at room temperature. It was noted that the sudden change from the room temperature to the outside temperature did not have any marked effect upon the existence of the organisms in the active condition.

PERIOD OF EXCYSTMENT OF SOIL PROTOZOA

Since active protozoa were not found in normal field soils, the question at once presented itself, How long a period of time was required for soil protozoa to become active in the presence of sufficient moisture, as, for instance, during a heavy fall of rain, and How long will they remain in the active state? In his work with *Colpoda cucullus* Goodey (3) in 1913 found that at 30° C. many were active after an hour. It was suggested by Martin and Lewin (8), as previously noted, that they may become active in a few minutes. To prevent misunderstanding as to the presence of motile protozoa in the soil, the writer in his method of examination proposed a 2-minute examination of each sample—i. e., the soil was in contact with free water no longer than two minutes at each examination. In no case during the entire course of the many examinations of field soils were any protozoa noted to have excysted

during the 2-minute examination, for in no case were any living protozoa found. It was later found with a limited number of soils examined that no protozoa were observed to excyst in a 5- or even 7-minute period. More evidence on this point is being collected.

Some evidence as to the length of time required for the excystment of soil protozoa when sufficient moisture is available is presented in Tables II and IV. As shown in Table IV, at the incubation temperatures of 5° to 7° and 32° to 33° a few small ciliates and flagellates were observed 8 hours after the increased additions of water were made to the soils. It is also seen that in nearly all samples incubated at 15° to 17°, 22° to 24°, 32° to 33°, and at outdoor temperatures some motile protozoa were present after 30 hours. The higher temperatures seemed to be more favorable for the more rapid excystment. This was also found to be true (1) when protozoa were developed in artificial-culture solutions. Small ciliates excysted in as short a period as did the flagellates. In Table II it is shown that after the soils had been in contact with water for 40 hours all of them showed the presence of small ciliates and flagellates. In several samples active large ciliates were also observed.

In order to accumulate more data as to the period of excystment of protozoa a small sample of each of the three soils (samples air-dried and samples containing an optimum amount of moisture and incubated at 22° to 24°, as given in Table III and in the text just following Table III) were added to a few drops of sterile tap water on a glass slide with a large depression in the center. The soil was stirred with a stirring rod and the film spread over the surface of the slide. A careful examination of each sample was made for a period of five minutes, and the slides containing the samples were then placed in the incubator. They were again examined for 5-minute periods at intervals of 15 minutes and 1, 2, 3, 5, 6, and 8 hours. (See Table VI.)

TABLE VI.—*Time required for the excystment of soil protozoa at 22° to 24° C.*

Laboratory No.	First examination.	After 15 minutes.	After 1 hour.	After 2 hours.	After 3 hours.	After 5 hours.	After 6 hours.	After 8 hours.
I22I.....	F.††
I223.....	F.†.....
I32I.....
I323.....	S.C.†..	S.C.†..	S.C.†..
I42I.....
I423.....

^a S. C.†= few small ciliates; F.†=few flagellates; F.††=several flagellates.

From the data recorded in Table VI it will be noted that at 22° to 24° protozoa (small ciliates) may excyst within two hours after the protective cysts come in contact with available moisture. Flagellates and other

small ciliates are seen to excyst in from six to eight hours after the immersion of the cysts in water. From the limited amount of study given to this point no conclusive statement as to the relative length of time required for the excystment of soil protozoa can be made. Nevertheless, the writer is of the opinion that under normal conditions protozoa excyst seldom, if at all, in as little as two minutes. There may be cases, however, as where the protective cyst is partially ruptured either by mechanical means or otherwise or where the moisture conditions are almost favorable enough for excystment, in which the organisms will become active in less than two minutes; but under ordinary normal conditions it seems doubtful from the examinations already made whether they can become active in this period of time at 22° to 24°. The indications (Table IV) are that excystment goes on more rapidly at higher temperatures. In all probability the original moisture content of the soil plays a part in determining the length of time which must elapse before the organisms become active. Likewise, different types of protozoa will prefer different conditions (1) and may excyst sooner at one temperature than at another. Further study on this point will be made.

SUMMARY

Under the conditions recorded in this paper the following observations as to the activity of soil protozoa seem to be justified:

(1) Under ordinary greenhouse conditions small ciliates, flagellates, and amebæ are active in some soils, but their presence is very limited.

(2) Active protozoa (small ciliates, large ciliates, flagellates, and amebæ) do not seem to be present in field soils with a normal moisture content and even when the moisture content is slightly supernormal, and, hence, they would not be a limiting factor in the soil.

(3) All field soils contain cysts of protozoa the organisms of which become active when conditions become favorable.

(4) The moisture content of the soil is the primary influencing factor which determines the presence or absence of the active protozoa in the soil, while the temperature, the presence of organic matter, and the physical properties of the soil are secondary factors.

(5) Soon after standing water is accumulated, as after a heavy rain, some protozoa will excyst and be active as long as the moisture content is favorable. Active protozoa seem to be always present in free standing soil water.

(6) Active protozoa are present in water-logged soils at constant and variable temperatures.

(7) Under normal conditions it would seem that protozoa can not excyst in 2 minutes. Small ciliates can excyst in 1 to 2 hours at 22° to 24° C.; at the same temperature flagellates can excyst in 6 to 8 hours and large ciliates can excyst in 40 hours.

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BERIBERI AND COTTONSEED POISONING IN PIGS¹

[PRELIMINARY NOTE]

By GEORGE M. ROMMEL, *Chief, Animal Husbandry Division, Bureau of Animal Industry*, and EDWARD B. VEDDER, *Captain, Medical Corps, United States Army*

SO-CALLED COTTONSEED POISONING OF ANIMALS

Cottonseed meal is one of the most valuable feedstuffs at the command of the American stockman. After the animal has digested it, the value of the residue as fertilizer is about three-fourths the original value of the meal. The United States uses only part of the cottonseed meal which it produces, and one of the reasons which prevent a larger domestic consumption of this by-product of the cotton industry is the danger that sickness and death may follow its use.

Cattle fed for more than 90 to 120 days on a heavy cottonseed-meal ration (6 pounds or more per head daily) become lame, and their eyes discharge freely, blindness often resulting. Deaths may occur, especially in young animals. Pigs are peculiarly susceptible to the effects of cottonseed meal, possibly because they are usually fed a larger quantity of the meal in proportion to their body weight. In feeding pigs, symptoms of sickness may appear at any time after three weeks of feeding, and deaths frequently occur with little warning.

Various systems of feeding cottonseed meal to pigs have been devised. Some of them appear to minimize its danger somewhat, but none of them prevent it entirely. This product, therefore, can not be regarded as a safe feed for pigs in the combinations in which it has heretofore usually been fed.

Among the more pronounced symptoms observed in pigs suffering from the effects of cottonseed-meal feeding are diarrhea; a harsh, rough, curly coat; paralysis; and shortness of breath. Emaciation and dropsical conditions are frequently observed. The disease manifests two forms—acute or chronic.

The acute form is much more serious to the farmer, because pigs are attacked by it with little warning and may be dead before any indications of disease are noticed. The largest and best nourished pigs are often the ones attacked. The attack is sudden and sharp. The pig experiences extreme shortness of breath and suffers the most intense pain. If he recovers, recurrences of the attack are likely, especially if the pig is a heavy feeder. Subsequent attacks may end fatally, or the disease may assume the chronic form.

¹ This opportunity is taken to express appreciation of the cooperation of Dr. Adolph Eichhorn, Chief of the Pathological Division of the Bureau of Animal Industry, in having made the necessary post-mortem examinations of pigs used in these experiments.

In the chronic form fatal results may not occur for a considerable time. The symptoms persist if the feed is not changed, and the pig appears to develop a certain degree of immunity to the effects of the disease. His condition, however, is continually, although slowly, declining. Pigs suffering from this form of the disease may live for a year or more on a cottonseed-meal ration.

On post-mortem examination, pigs which have died from the effects of cottonseed-meal feeding show large quantities of fluid in the abdominal and thoracic cavities and in the pericardial sac. The kidneys, liver, spleen, and small intestines are usually congested. In some cases the membrane lining the stomach is eroded. The lungs are very edematous, especially in pigs which have died from sudden acute attacks. The heart is enlarged.

SIMILARITY OF SYMPTOMS OF COTTONSEED POISONING AND OF BERIBERI

These conditions bear a striking resemblance to those seen in the disease known as beriberi in man, which, according to Vedder,¹ results "from faulty metabolism * * * and is directly caused by the deficiency of certain vitamins in the food."

Beriberi in human beings is usually caused by a diet of highly milled rice and is never known to result from a diet of rice from which the pericarp and aleurone layer of the grain have not been removed. However, the disease may be caused by diets of which rice forms no part whatever. For example, a diet of bread or macaroni alone made from highly milled wheat flour will produce beriberi. Birds (chickens and pigeons) are generally used in the laboratory study of beriberi because they readily develop the chronic or "dry" form when fed on a diet of highly milled rice for a sufficient time, but they will also develop the disease if fed on an exclusive diet of white wheat bread.

Beriberi in pigs is not frequently reported in the literature on the subject. Braddon² reports, without details, the case of a pig fed on polished rice. The pig developed paralysis in about a month and died suddenly. It is believed that until this year this was the only case of the kind recorded.

EXPERIMENTS TO COMPARE EFFECTS OF FEEDING POLISHED RICE AND COTTONSEED MEAL

On August 31, 1915, the writers began a series of experiments to determine (a) whether the "wet" or acute form of beriberi could be produced in pigs on a diet of polished rice, and (b) whether the disease heretofore called "cottonseed poisoning" in pigs is not really beriberi.³ Four pigs

¹ Vedder, E. B. *Beriberi*. p. viii. New York, 1913.

² Braddon, W. L. *The Cause and Prevention of Beri-Beri*. p. 355. London, New York, 1907.

³ It should be noted that Withers and Carruth made no extensive use of pigs in their investigations on gossypol. (Withers, W. A., and Carruth, F. E. Gossypol, the toxic substance in cottonseed meal. *In Jour. Agr. Research*, v. 5, no. 7, p. 261-288, pl. 25-26. 1915.)

were fed a ration of 9 parts (by weight) of steamed polished rice and 1 part of tankage, and four a ration of 2 parts of corn meal and 1 part of cottonseed meal. On October 24 the ration of the latter pigs was changed to equal parts by weight of corn meal and cottonseed meal. None of these pigs had received rice or cottonseed meal before they entered the experiment.

On September 8 one of the pigs on rice began to breathe with difficulty. On the 10th this condition was pronounced, and he refused to eat. On September 14 these symptoms rapidly became more severe, paralysis developed, and the pig died shortly before noon. The ante-mortem symptoms were what one would expect to see in an acute case of so-called cottonseed poisoning. They were, in fact, the symptoms of wet beriberi. The post-mortem examination showed serous fluid in the pericardial sac and in the thoracic and abdominal cavities. The heart was enlarged and the cardiac muscle congested. The lungs were decidedly edematous and mottled with a fair number of small subpleural hemorrhages. The liver was intensely congested and enlarged. The spleen was apparently unaltered, but was dark in color. The stomach showed several erosions in the mucosa, and the walls were thickened. The small intestines were slightly congested. Many of the mesenteric glands were enlarged and congested. Both kidneys were congested, especially at the apices, which were deep cherry-red in color. The bladder was distended with urine, which contained a large amount of albumin. Except for the large quantity of albumin, this is exactly what one would expect to find in a beriberi necropsy. It is also what is found in an acute cottonseed-meal necropsy.

On September 21 four additional pigs were placed on the same steamed rice and tankage ration (9:1). On September 29 one of these pigs became sick and on September 30 it refused to eat. He recovered and regained his normal appetite, but died on October 29, after having been on the rice diet for 38 days. The ante-mortem symptoms corresponded closely to those of the first pig to die, but the post-mortem examination did not give such clear-cut results. The sciatic nerves of this pig were dissected out immediately after the post-mortem examination and, after being treated by the Marchi method, showed considerable degeneration of the nerve fibers.

The writers believe that pigs fed a ration in which rice is the chief component will develop beriberi as do human beings, but much more quickly. Weight is given to this belief by the experience of Moore,¹ who lost pigs fed on "rice meal"² from a disease which Hadwen³ suspects to be beriberi.

¹ Moore, P. H. Hog-feeding experiments. In *Canada Exp. Farms Rpts.* [1912]/13, p. 611-613. 1914.

— Preliminary note on the effects of feeding rice meal to pigs. In *Canada Dept. Agr. Rpt. Vet. Dir. Gen.* [1913]/14, p. 137-141. 1915.

² Apparently not the rice meal of our Southern States.

³ Hadwen, S. Notes on the pathology and symptoms of rice-meal fed pigs. In *Canada Dept. Agr. Rpt. Vet. Dir. Gen.* [1913]/14, p. 140. 1915.

The remaining 10 pigs are being continued on the rice and cottonseed-meal rations. At the time this article is written they have been almost 90 days on these feeds. All the pigs are sick, and the same symptoms have appeared in each lot. In fact, it may be said that the most typical and acute cottonseed-meal symptoms are seen among the pigs receiving rice.

A mature brood sow, weighing 400 pounds, due to farrow on November 14, 1915, was placed on a cottonseed-meal ration on September 2. She was started on a ration of 4 parts of corn meal and 1 part of cottonseed meal, the quantity of corn meal being gradually decreased until, on October 1, she was receiving equal parts of corn meal and cottonseed meal. Up to November 14 she had eaten 134.65 pounds of cottonseed meal. She showed no serious sign of sickness, except nausea on November 4, when she vomited. At 8 p. m. on November 13 she began to farrow and delivered 9 pigs, the last one being born at 4 o'clock the following morning. Four of these pigs were born dead, and of those born alive all but one died in a few minutes. The last pig born lived less than eight hours.

Post-mortem examinations were made of seven of these pigs, four of which had been born alive. All of them showed enlarged hearts, and serum was found in the pericardial sac, the thoracic cavity, and the abdominal cavity. The quantity of serum was a little greater in the pigs born alive than in those born dead. In the pigs born alive there was some injection in the lungs, liver, and small intestines, but none in those born dead. There were no alterations in the kidneys of any of the pigs born alive or dead.

These pigs were very well developed, plump, and apparently had been well nourished. They averaged slightly over 2 pounds 6 ounces in weight. The analogy with infantile beriberi is apparent. Yet the dam had never eaten rice, and the only assignable cause for the death of her litter was the cottonseed meal in her ration. Her breeding record for previous farrowings is as follows:

Item.	1914	1915
Date of farrowing.....	Apr. 7	Apr. 2
Number of pigs.....	5	12
Number born alive.....	5	9
Number raised.....	4	5

The sow was a good breeder, and difficult labor can not be given as the cause of the death of the litter.

CONCLUSIONS

The studies of the writers seem to lead to three general conclusions:

(1) Pigs are susceptible to beriberi when fed on vitamine-deficient rations, such as rice. The disease develops much more rapidly in pigs

than in man. In man symptoms rarely, if ever, appear before 90 days. In pigs the writers have found symptoms of a pronounced character in from 8 to 10 days.

(2) It is believed that the so-called cottonseed poisoning of pigs is a deficiency disease, analogous to the disease known as beriberi in man, if not indeed identical with it. Acute cottonseed poisoning corresponds to wet beriberi, and the chronic form to dry beriberi.

(3) The cause of the so-called cottonseed poisoning is probably a deficiency in the ration, causing, among other manifestations, profound changes in the nervous system.

At first thought this theory is not justified. Beriberi results from a ration of highly milled rice, because substances vitally necessary to the animal organism have been removed from the rice grain in the process of milling. When pigs suffer from so-called cottonseed poisoning, it is only when cottonseed meal has been added to the ration. Pigs are seldom, if ever, fed on cottonseed meal alone.

The following explanation of this condition is offered: The grain with which the cottonseed meal is most frequently combined is corn. Corn is notoriously deficient as a single feed for animals, and it must be properly balanced to be fed satisfactorily. The excellent results in feeding pigs which can be obtained from rations of corn meal and skim milk or other animal products, such as tankage, blood meal, fish meal, etc., are out of all proportion to the facts indicated by the conventional chemical analyses of protein, carbohydrates, and fat. When corn meal is fed with cottonseed meal, a combination is made of two feeds both of which are deficient.

The writers are engaged in further studies of this subject to determine more exactly the effects of cottonseed meal when fed in the ration of the pig, and to determine whether methods similar to those used to prevent beriberi in man can be practically applied to prevent the so-called cottonseed poisoning of pigs.

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BIOLOGY OF APANTELES MILITARIS

By DANIEL G. TOWER,¹

Scientific Assistant, Cereal and Forage Insect Investigations,
Bureau of Entomology

INTRODUCTION

The results herewith presented deal with *Apanteles militaris* Walsh, a braconid endoparasite of the army worm (*Heliophila unipuncta* Haw.). The series of experiments on which the main part of this paper is based was begun on September 29, 1914, at La Fayette, Ind. They were carried on in the laboratory, the parasitized caterpillars being kept in glass vials plugged with cotton and fed fresh corn leaves as required. The laboratory windows were left open, so as to make conditions as nearly like those outside as possible. During the few cold days which were experienced the laboratory was heated to the normal room temperature. During the first two weeks in August additional records were kept of the time spent in the cocoon by the parasites, and in these experiments cocoons were kept in tin salve boxes in an outdoor insectary. On November 16 a series of experiments was started indoors to determine whether or not this species is parthenogenetic, and conclusive results were obtained. The caterpillars used in the experiments were raised from eggs unless otherwise stated.

DESCRIPTION OF LIFE STAGES

THE EGG

The egg measures 0.09 to 0.10 mm. in length and 0.025 to 0.028 mm. in width. It is rounded at one end, more or less pointed at the other, and slightly curved, the rounded end bearing a distinct micropyle. Subsequent swelling of the egg during the growth of the embryo causes

¹ The writer wishes to acknowledge his indebtedness to Messrs. J. J. Davis and A. F. Satterthwait, of the Cereal and Forage Insect Field Station of the Bureau of Entomology at La Fayette, Ind., for many helpful suggestions and material, and to Messrs. J. A. Hyslop and G. G. Ainslie, of the Bureau of Entomology, for their interest and kindness in collecting material and data for him at their respective stations. He is also indebted to Messrs. A. B. Gahan and W. R. Walton, of the Bureau of Entomology, for the determination of specimens and for the drawings of the three larval stages, respectively.

the point of the smaller end to assume the appearance of a nipple-like prominence.

The number of eggs laid by a single individual was not obtained, nor were the eggs in the abdomen counted, hundreds having been present.

EMBRYONIC DEVELOPMENT

The average length of the egg stage is $5\frac{1}{2}$ days. Individual records show that in some cases this may be shortened to $4\frac{3}{4}$ days or prolonged to more than $6\frac{1}{8}$ days. From the hundreds of developing eggs examined it was determined that only one larva hatches from each egg.

Development progresses rapidly within the egg. At first little can be distinguished, except that the egg becomes strongly curved, increases in size, and becomes more opaque, owing to the formation of the germ band. When the egg is ready to hatch it has increased in size from 0.09 or 0.10 mm. in length to 0.66 or 0.70 mm., and proportionally in width. This great increase in size can possibly be explained by the fact that the egg is probably deficient in nutritive matter when laid and that this is absorbed from the blood of its host by the developing embryo.

When embryonic development has progressed sufficiently to show the form of the embryo, this is seen to be surrounded by a single embryonic envelope one cell layer deep which, according to Korschelt and Heider (3, p. 287),¹ is the serosa (Pl. I, fig. 1). Whether the amniotic and serosal envelopes are at first separate has not been determined. According to Graber's observations on Hymenoptera, as reviewed by Korschelt and Heider, it would seem that the two envelopes are separate at first but later become indistinguishably united. At the time of hatching, a portion of the cells of this so-called serosal envelope are cast out at the poles of the egg (Pl. I, fig. 2) and become a body of loose cells lying between the chorion and the embryo (Pl. I, fig. 3), which is now tightly inclosed by a layer of broad, flattened cells made up of the remaining cells of the envelope (Pl. I, fig. 3). This rapid division apparently indicates that this envelope was the product of the fused amnion and serosa, which now separate at hatching time, the loose mass of cells being of serosal origin and the remaining thin envelope the amnion surrounding the embryo. Henneguy (2, p. 336-337), however, discusses insects that have only one embryonic envelope and lists among these parasitic forms, vegetable or animal, of the Cynipidae, Pteromalidae, and probably Ichneumonidae. It will be interesting to note whether other investigators observe this splitting of the single embryonic envelope at hatching time.

The mandibles can be seen forming at an early stage, and their chitination can be seen to progress until maturity is reached at hatching time.

¹ Reference is made by number to "Literature cited," p. 506-507.

The mouth opens into an enlarged cavity, the pharynx, this in turn opening posteriorly into a very narrow esophagus, and this into the stomach, which is a very long, narrow, tapering tube closed posteriorly. There are two Malpighian vessels, which lie parallel to the stomach, extending anteriorly about one-half the length of the larva.

The tracheal system has not been observed in the embryo. According to the observations of Weismann and Grasse, as reviewed by Korschelt and Heider (3, p. 334-335), the tracheal system forms early in the embryonic development of the Hymenoptera as compared with the lower forms of insects and usually contains air previous to hatching, this being obtained apparently from its tissues and body fluid. Seurat (7) states, however, from his study of *A. glomeratus*, that the tracheal system of this parasite, whose development is similar to that of *A. militaris*, is present, although he had not seen it, no doubt basing his statement on the fact that these organs, being ectodermal invaginations, are normally formed in the embryo.

The head of the mature embryo is of one segment and is readily distinguished by its large size, the presence of mandibles, two small tubercle-like antennæ, and the prominent brain lobes. A nervous system of 11 ganglia, not including the subesophageal ganglion, is visible. The segments of the body appear to be 10 in number, but subsequent development and growth in the first stage reveal 11 distinct segments.

The caudal vesicle, which in the larval forms is a large sac at the end of the body, is seen forming as a solid mass of long, narrow cells in the posterior region of the abdomen (fig. 1, *a*). When first seen it lies inside the abdomen, but can be seen gradually to grow out through the anal opening (fig. 1, *b*), which becomes greatly distended. The stomach becomes lengthened and extends outside the body into the vesicle, its blind end being fastened to the inside wall of the vesicle posteriorly and ventrally. The Malpighian tubes also extend into the vesicle and open through its ventral surface near the end of the stomach.

HATCHING

The embryo at the time of hatching, as previously stated, lies tightly inclosed in the amniotic envelope surrounded by the loose mass of serosal cells, the whole being surrounded by the chorion. The embryo, which up to this time has been curled in the egg, now straightens itself out and by its struggles to escape, aided by the rapid swelling of the serosal cells, ruptures the chorion, which has become extremely thin, owing to the increase in the size of the egg, and escapes into the body of its host, still

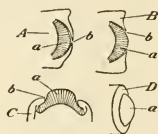


FIG. 1.—*Apanteles militaris*: A, B, C, Diagrammatic sectional views of the posterior end of the embryo, showing how the hypertrophied cells of the hind gut, which ultimately form the caudal vesicle, grow out through the anus. D shows an external view of this process. *a*, Mass of cells; *b*, anus. (Original.)

tightly inclosed in the layer of thin, flat cells. The serosal cells are scattered through the body of the host. The chorion shrinks and probably finally dissolves. The young larvæ are now 0.7 mm. in length and start feeding, after cutting through the amnion in the mouth region. At this time the mass of cells which forms the caudal vesicle has grown out through the anal opening.

THE LARVA

FIRST INSTAR (Pl. L, fig. 5).—The first larval instar averages $3\frac{1}{4}$ days, the first molt taking place, on an average, $8\frac{3}{4}$ days after oviposition.

The larva grows rapidly, increasing in length approximately from 0.7 mm. at hatching to 3.5 mm. at the first molt.

The head is made up of 1 segment and the body appears to have 10, but in subsequent growth the tenth segment divides into 2, making 11 in all. There are no spines or hairs on the segments, except a few in the oral region. Owing to the rapid growth of the larva, the embryonic envelope in which it is inclosed becomes ruptured and gradually falls off, although portions of it may remain until the first molt takes place. The mandibles are constantly in motion, attacking the fat body of the host. This, together with the blood, is the food of the parasites during this stage and is drawn in by means of a sucking pharynx. The alimentary tract does not change, except to increase in size, it being still further lengthened as the caudal vesicle expands.

Immediately following hatching, the slender cells of the mass which protrudes from the distended anal opening are compressed lengthwise, so that they become broad, flat cells, thus immensely increasing their exterior and interior surfaces, and there is formed at the end of the larva a large sac, the caudal vesicle, the walls of which are made up of a layer of broad, thin cells (Pl. L, fig. 5). The two Malpighian vessels are drawn out into the caudal vesicle, their relative positions being the same as in the embryo.

The origin of this caudal vesicle and its functions in the two endoparasitic stages will be considered later.

The nervous system appears as in the embryo, its growth keeping pace with the growth of the larva.

No tracheal system is visible during this instar.

The heart can be seen forming in the early part of this instar. It lies dorsally and has nine pairs of valves, its lateral controlling muscles being readily seen. Anteriorly it narrows to an aorta which opens into the posterior region of the head. Instead of ending normally in the posterior end of the body, a rudimentary tube lying dorsally in the caudal vesicle connects with the heart (Pl. L, fig. 4). This tube extends posteriorly, opening in the dorsal posterior region of the caudal vesicle, and forms a channel through which the blood is sucked into the heart. When the heart commences to function, which it does during this stage, the blood,

having been drawn through the rudimentary tube into the heart, is there passed along by a series of wavelike motions into the head, the valves preventing the return of the blood. From here it circulates through the body in returning to the caudal vesicle, the walls of which it bathes before starting on a new cycle. A careful examination of the heart does not show that ostia are present; hence, the blood necessarily follows the course described above.

The silk glands can be distinguished early in this stage and lie on either side of the stomach as two straight tubes which meet anteriorly in the head and extend to the spinneret. As the end of this stage approaches, these glands begin to coil, taking on a wavy appearance.

SECOND INSTAR (Pl. I, fig. 6).—The second instar averages $5\frac{1}{2}$ days, terminating when the larva emerges from its host, for it molts at this time. During this stage the average increase in length is from 3.5 to 6 mm., although when a great many larvæ are present in a host their size may be reduced nearly one-half. The caudal vesicle normally during this stage reaches the length of 1 mm. (Pl. I, fig. 6).

The head of the larva is made up of 2 segments. The anterior one bears a few spines about the oral region and is much smaller than the posterior and almost wholly retractile in it. There are no notable characters or ornamentations on the segments of this larva. The body has 11 segments and is at first slightly darker than the first instar, but rapidly becomes more so as the fat body accumulates. The mouth parts are not developed, nor are those of the third instar ready for use, until the larva is ready to emerge from its host; hence, it is seen that only the blood and the solid matter contained in it are used for food during this stage. In older forms there are 7 hyaline areas protruding on each side of the body lying between the segments.

The silk glands grow rapidly, becoming more and more coiled and twisted, and are readily seen lying on either side of the alimentary tract, nearly filling the body cavity.

The heart and the circulation of the blood are the same as in the first instar.

The nervous system consists of the supraesophageal and the subesophageal brains and 11 ganglia with their branches, as in the first instar. In the early life of this stage the imaginal discs of the compound eyes are noticeable and appear to be in the first thoracic segment. The exhaustive studies of Seurat (7) show clearly that although other authors have thought that a portion of the prothorax entered into the composition of the head of the pupa, it is formed only from the head of the larva and that in the larval forms a portion of the head has simply been thrust back into the prothorax. Ventrally in the thoracic segments the three pairs of imaginal discs of the legs are present, and laterally in the mesothorax and metathorax those of the wings can be seen.

The mouth, pharynx, esophagus, and stomach have approximately the same form and relative positions as in the first instar. Owing to the fact that the blood of the host is green, the stomach content of the parasite at first takes on a greenish brown color which finally becomes a deep green, similar to the blood of the host, and later, at the end of the stage, this again becomes greenish brown.

During the last two days of this stage the anal opening, the diameter of which nearly equals that of the body, slowly contracts, and violent contractions of the longitudinal muscles of the stomach, which cause it to shorten, slowly draw the caudal vesicle in through the contracting anal opening. The Malpighian vessels are also drawn in by the contraction of the stomach and are now two-thirds as long as the larva. After the caudal vesicle has been drawn completely within the body, the anal opening contracts still further, and the anus is formed.

The tenidia of the tracheal system can be seen forming soon after the first molt. Those of the two main longitudinals and their anterior connecting branches are first visible, and there are 11 branching centers on each longitudinal from which arise branches sending tracheæ to all parts of the body, some even extending posteriorly into the caudal vesicle along the lateral walls and the stomach. Nine pairs of short, stublike branches are noticeable in the older larvæ, arising near the bases of the anterior nine pairs of dorsal branches of the main longitudinals. In the still older larvæ, those nearly ready to emerge, eight pairs of spiracles can be seen forming at the surface of the body, and these are connected with the first, and the third to ninth, inclusive, pairs of stublike branches previously mentioned, by tracheæ destitute of air. These become filled with air when the larva molts at emergence and the spiracles are uncovered and function. The spiracles that connect with the second pair of stublike branches do not form during this stage.

After the caudal vesicle has been drawn in, the larva is ready to emerge from its host. The mandibles of the third instar, which are now developed and protrude, slowly cut and tear through the muscles and skin of the host as the larva presses its head against the body walls of the caterpillar and moves them backward and forward. When a slit has been made of sufficient size the larva squeezes through the opening, molting the previously loosened skin as it emerges. During this process the caterpillar lies quietly as though paralyzed. About the time the parasites have nearly finished their cocoons, it usually revives enough to crawl away.

THIRD INSTAR (Pl. I, fig. 7).—The third instar lasts from the emergence from the host until pupation, the time being approximately $2\frac{1}{2}$ days.

The newly emerged larva is light green in color. It is covered with minute spines, with a number of short black spines somewhat irregularly

placed on the segments; also about the mouth there are a few hyaline spines. The brown-colored compound eyes are very noticeable and appear to be, as in the second stage, in the prothorax. The segmentation of the body is apparent and the eight pairs of spiracles are plainly visible (Pl. L, fig. 7). The mouth parts are at the extremity of the head and are composed of labrum, mandibles, maxillæ (bearing the rudimentary maxillary palpi), and the labium (bearing the rudimentary labial palpi). Laterally the seven hyaline protruding areas form an irregular, conspicuous, longitudinal ridge on either side of the body.

When the larva has emerged for about two-thirds of its length, it stops and commences to spin its cocoon. The silk comes from the two orifices of the spinneret situated at the base of the labium. The cocoon is spun in two parts, the outer part loosely and the inner compactly. The first few threads spun are fastened to the ventral side of the body, after which a series of large loops are made, the silken thread being drawn out and fastened to the top of the loop below. These extend up the ventral side laterally and over the head of the larva as far back as it can bend. The larva now draws its anal end out of the host, reverses its position in the partly spun outer cocoon, and spins the remaining side and end. The inner or thin, dense cocoon is now spun by a series of long, narrow, longitudinal and diagonal loops. The tough silken cocoon is encircled near one end, or sometimes at both, by a thinner, narrow area, through which the adult parasite easily cuts, removing a caplike portion, the end of the cocoon, as it emerges.

At the end of the first day or the beginning of the second the connection between the stomach and proctodeum is opened and the accumulated waste is voided, being deposited at the anal end of the cocoon. When pupation takes place, the last larval skin is molted and pushed to the anal end of the cocoon and lies over the waste. Previous to pupation, the constriction between the thorax and abdomen, which results in the cephalization of the first abdominal segment, is distinctly seen.

PUPA AND ADULT

The pupal stage averages from $8\frac{1}{2}$ to $9\frac{1}{2}$ days.

The pupa is light cream yellow and lends the same color to the cocoon. The eyes and ocelli appear as brown spots. Later, the chitin in the head and thoracic region commences to darken, closely followed by that of the abdomen. When the adult becomes active in the cocoon, the pupal skin is kicked off, and the area of thin silk is cut through by the mandibles, the end, or cap, of the cocoon being pushed off by the emerging adults. As soon as the adult is out of the cocoon, it passes a quantity of waste, cleans itself, and straightens and dries its wings.

LENGTH OF LIFE CYCLE

The total length of the life cycle, as obtained in the series of experiments carried from the last of September to the last of October, averaged 25 days. A series of experiments conducted during the first two weeks of August to determine the time spent by the third instar and pupa in the cocoon varied from 5 to 7 days, as compared with 11 to 12 days during September and October. This great reduction in the time spent in these periods of development raises the question whether or not the time spent in the host would not be shortened under summer conditions. Unfortunately, this point could not be determined; but considering that the duration of the larval life of the army worm varies from 20 to 30 days, according to Slingerland (8), it seems not unlikely that the length of the egg and internal larval stages would vary correspondingly with the life of the host.

COPULATION

The following observations were made on these insects confined in test tubes and lantern-globe cages. The male pursued the female, caressing her with his antennæ, often mounting her posteriorly and, thrusting his abdomen forward, bringing the ventral surface in contact with that of the female. Once union had taken place the male folded his wings and drew his legs close to his body, holding on to the female solely by his genitalia. It was noticed that in the case of a number of males and females confined in test tubes for several days, copulation continued to take place day after day with unabated vigor.

OVIPOSITION

The parasite apparently recognizes the host on touching it with its antennæ, and following such recognition the ovipositor is bent beneath the thorax, sometimes slowly but usually quickly, and is then rapidly thrust into the caterpillar. This being done, the parasite folds its wings and draws its legs up close to its body, holding on to the caterpillar solely by its ovipositor, this no doubt being done to protect itself from the attacks of its host. During the process of oviposition the caterpillar may throw itself about violently, but rarely dislodges the parasite.

Of the number of apparent ovipositions in larvæ of the third, fourth, and fifth stages, one-sixth of those which took place in the third, one-fifth of those in the fourth, and one-half of those in the fifth stage were unsuccessful. Usually the parasite larvæ emerge after the caterpillar is full grown, as observed in the case of larvæ collected in the field and those parasitized in the laboratory under artificial conditions, but in one instance where the parasite oviposited in a caterpillar of the third stage the parasite larvæ issued during the fifth stage.

Parasites readily attempted to oviposit in caterpillars of the fifth and sixth stages, but were apparently unsuccessful, on account of the tough-

ness of the skin, except in newly-molted fifth-stage larvæ. In such cases they would run along the back of the host, jabbing with the ovipositor but never succeeding in puncturing the skin.

The eggs, when dissected from the body of a caterpillar immediately following oviposition, are found to be separate.

Oviposition in the field under natural conditions resulted in the following numbers of cocoons collected from single hosts: 56, 90, 71, 79, 90, 7, 113, and 66. In the laboratory from 8 to 72 eggs were deposited in one oviposition of less than one second, and in one case of four ovipositions 210 eggs were deposited in the same host. The extreme rapidity of oviposition is apparently due to the activity of the caterpillar, which usually immediately recognizes its enemy, rapidly smearing her with saliva and often biting her.

PARTHENOGENESIS

During November and December a number of experiments were conducted in the laboratory to determine whether parthenogenesis takes place. Unfertilized females were obtained from separate cocoons and were allowed to oviposit in small caterpillars, which they readily did. Males emerged from all the cocoons of *A. militaris* originating from these caterpillars, clearly showing that this species is parthenogenetic and indicating that unfertilized females give rise to a generation of males.

FEEDING EXPERIMENTS AND LONGEVITY

Adults which emerged on August 14 were confined in a lantern-globe cage in which grass was growing. They were fed on a mixture of honey and water, this being sprayed in minute droplets on the grass and walls of the cage. The adults were of both sexes and were kept alive for some time, the last one dying on September 1.

One female used in oviposition experiments was kept alive for eight days in a test tube, being fed honey, and another under the same conditions lived for seven days.

On November 6 and 7 a large number of newly emerged males were confined and fed in two lantern-globe cages indoors, as described above. These males were not allowed to copulate, and many lived until the first of December, the last dying on December 9 and 10.

WINTERING FORMS

All attempts at this station (La Fayette, Ind.) to winter this parasite under various conditions while in the cocoon have been unsuccessful. Mr. G. G. Ainslie, stationed at Nashville, Tenn., found this year (1915) that the army worm passed the winter there as young larvæ and, further, that specimens under observation were parasitized in the fall, for the parasites completed their growth and emerged this spring. Again, according to Gibson (1, p. 27), the army worm winters in Canada as

young larvæ beneath tufts of grass. Considering the data at hand, the theory is advanced that in the North the parasites winter as partly developed forms in immature larvæ, while in the South they no doubt also winter while in the cocoon.

ORIGIN AND FUNCTION OF THE CAUDAL VESICLE

The following is a summary of the results of the studies of Weissenberg and Seurat, together with the observations made by the writer, on the origin and function of the caudal vesicle, obtained mainly from experiments with hymenopterous endoparasites.

As Seurat's (7) and Weissenberg's (9) papers both deal with *A. glomeratus*, the caudal vesicle of which originates and functions identically as does that of *A. militaris*, the results of their studies are applicable to *A. militaris*. Weissenberg's paper, being the more exhaustive and, in addition, containing studies of the larva of this parasite in comparison with others less highly specialized, is used as a basis for this summary.

Observing the beginning of growth and the subsequent expansion of the caudal vesicle, the writer supposed that the entire proctodeum evaginated and turned inside out, but the careful histological studies of *A. glomeratus* by Weissenberg show that only a portion of the proctodeum through rapid growth becomes specialized to form the vesicle, while the remainder becomes temporarily atrophied. According to Weissenberg, the vesicle is formed by the rapid growth and elongation of the cells of the proctodeum which form the posterior end of the plug at the posterior end of the stomach, together with those adjacent cells at the anterior end of the proctodeum which surround the opening of the larval Malpighian tubules and extend posteriorly a short distance to the rudiments of the adult Malpighian tubules. The mass of elongated cells thus formed grows out through the anal opening of the embryo, and immediately following hatching these elongated cells are compressed lengthwise, so that their long axis becomes their short one, resulting in broad, flat cells joined edge to edge to form the thin wall of the caudal vesicle. During the rapid growth of these cells in the pyloric region the remainder of the proctodeum becomes atrophied and stays so until the caudal vesicle is drawn in. At this time parts specialized for endoparasitic life are reduced, and the atrophied parts grow rapidly, the whole approaching the normal proctodeal development of a free-living hymenopterous larva, previous to pupation.

Weissenberg next compares the origin and cellular structure of the caudal vesicle of *A. glomeratus* with that of the caudal appendage of the endoparasitic larval form of an undetermined species of *Macrocentrus*, and shows them to be homologous. In *Macrocentrus* sp., however, the cells always remain as a mass of long, slender cells protruding through the anal opening, a vesicle never being formed. The early stage of the

species of *Macrocentrus* studied was equipped with a tracheal system, while the corresponding stage of *A. glomeratus* was not. The conclusion is drawn that the vesicle functions as a blood gill in *A. glomeratus*, since all the blood necessarily pours through this vesicle, bathing its walls, while in *Macrocentrus* sp., which possesses a tracheal system, such an adaptation is not necessary.

An unknown species of the genus *Limneria*, parasitic on *Plutella cruciferarum* Zell., is next introduced for comparison by Weissenberg. In this parasite the portion of the proctodeum homologous with those of the two preceding larvæ discussed is not so well developed, for while pseudopod-like structures extend into the anal lumen, they do not protrude through the anal opening, which, however, is nevertheless very large. In this species it is clearly shown that the cells of these pseudopod-like structures completely correspond histologically with those of the larval Malpighian tubules. In a similar manner these specialized portions of the proctodeum of the two species last discussed are reduced and the portions retarded grow rapidly, approaching the normal proctodeal development of free-living larvæ before pupating, the normal proctodeal development of *Hemiteles fulvipes*, an ectoparasite of *A. glomeratus*, being used in comparison to illustrate this.

In the last analysis it is seen that the cells of these proctodeal appendages of the three endoparasitic larvæ considered are histologically allied with the cells of the larval Malpighian vessels, and with this in mind Weissenberg brings out clearly the idea that these proctodeal organs have also an excretory function and credits Kulagin (4, 5) with first suggesting this from results obtained from his injection experiments. Weissenberg further thinks that the excretory apparatus has undergone a superficial enlargement, owing to the active metabolism characteristic of this group, and that as excretory products in general are poisonous, it would seem natural to find here an adaptation by which they can be eliminated. His concluding argument is that in *A. glomeratus*, *Macrocentrus* sp., and *Limneria* sp. the development of the larval Malpighian vessels forms an ascending series, they being only rudimentary in *A. glomeratus* in comparison with the well-developed ones found in *Limneria* sp., while the proctodeal adaptations form a descending series, being most highly specialized and developed in *A. glomeratus* and only partly so in *Limneria* sp.

From the facts presented above and this study of *A. militaris*, the author concludes that the caudal vesicle is primarily an excretory organ and that the function of respiration is secondary. The following observations seem still further to strengthen this conclusion. The caudal vesicle functions from approximately the beginning of feeding to its close, and the portion of the first skin molted which covers the vesicle becomes greatly swollen in the second stage with a liquid content until finally it is ruptured. Further, the food of the larva is mainly the already digested solid parts

of the blood of the host, these being retained in the stomach during endoparasitic life, while the liquid parts, which are in excess, together with the by-products of anabolism and katabolism formed in the body of the rapidly developing larva, are eliminated by means of this enlarged adaptive excretory organ, which is bathed by the blood at each cycle. These by-products are doubtless eliminated from the body of the host, as are its own, by the Malpighian vessels. The caudal vesicle no doubt respire, this action taking place by osmosis, as is generally considered to be the case in endoparasites having a closed tracheal system. Whether respiration is more rapid through the walls of the caudal vesicle or whether they are especially adapted for it can not be positively stated, although Weissenberg, as stated previously, thinks that the vesicle functions as a blood gill. Again, that this portion of the body wall of the larva is apparently the thinnest and least chitinized is quite evident; therefore, it would not seem unreasonable to suppose that respiration takes place to a large degree through this area and that the air is carried mechanically throughout the body of the larva by the blood and is taken up from it to fill the closed tracheal system when it develops in the second instar.

Seurat's theory (7) that the essential function of the caudal vesicle is that of locomotion is no doubt incorrect, for careful observations of the movements of the larva show that the vesicle, because of its large size, is actually a hindrance to the larva in moving about in its host. Weissenberg (9) has also shown that the caudal vesicle is not homologous with the tail-like organs generally considered to be locomotor appendages which occur in various endoparasites, for both these organs are present in the larva of *Macrocentrus* sp. studied.

An additional point brought out by Weissenberg is that the caudal vesicle is an adaptation of the biophagous larva for its mode of life, for the necrophagous larva does not have it, and these adaptations arise from a biophagous mode of life in contrast with the necrophagous rather than from an endoparasitic life in contrast with an ectoparasitic life, as has been previously supposed.

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PLATE I

Apanteles militaris:

Fig. 1.—Diagrammatic drawing showing the embryo inclosed by the fused amniotic and serosal envelopes. *as*, Fused envelopes; *c*, chorion; *cv*, caudal vesicle; *h*, head.

Fig. 2.—Diagrammatic drawing showing the fused envelopes dividing into their two parts, the serosal cells being grouped at each pole. *a*, Amnion; *c*, chorion; *cv*, caudal vesicle; *h*, head; *s*, serosal cells.

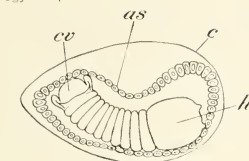
Fig. 3.—Diagrammatic drawing showing the egg ready to hatch, the serosal cells having become a loose mass and the embryo straightened out in the egg. *a*, Amnion; *c*, chorion; *cv*, caudal vesicle; *h*, head; *s*, serosal cells.

Fig. 4.—Diagrammatic drawing of the larva during its first molt. *b*, Brain lobes; *cv*, caudal vesicle; *h*, head; *ht*, heart; *m*, molted skin; *mp*, Malpighian tubes; *o*, esophagus; *p*, pharynx; *sg*, silk glands; *st*, stomach; arrows indicate the blood cycle; *t*, rudimentary tube in the caudal vesicle connecting with the heart.

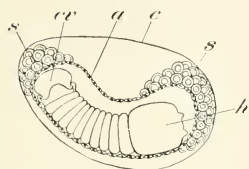
Fig. 5.—First instar. *cv*, Caudal vesicle.

Fig. 6.—Second instar. *cv*, Caudal vesicle.

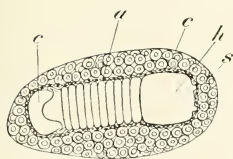
Fig. 7.—Third instar, showing the position of the spiracles and the caudal vesicle withdrawn.



1



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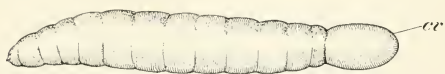
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RESPIRATION EXPERIMENTS WITH SWEET POTATOES

By HEINRICH HASSELBRING and LON A. HAWKINS,

*Plant Physiologists, Drug-Plant, Poisonous-Plant, Physiological, and Fermentation
Investigations, Bureau of Plant Industry*

INTRODUCTION

In 1882 Müller(7),¹ in the course of his classical researches on the accumulation of sugar in plant organs at low temperatures, observed that potatoes (*Solanum tuberosum*) which had been kept for a time at 0° C., and whose sugar content had in consequence been greatly increased, respired much more energetically than potatoes of lower sugar content. Even before the experiments of Müller, a number of analogous facts were known, all indicating that the respiratory energy of plants is a function of their carbohydrate content. Thus, isolated rootlets and seedlings deprived of their cotyledons show a rapid decrease in their respiration on account of the lack of plastic material normally furnished by the cotyledons (12). In etiolated seedlings the respiration curve rises at first as the food substances in the cotyledons or endosperm become available, and after passing a maximum falls gradually with the exhaustion of the food reserve (6, 11). The respiration of isolated leafy shoots kept in the dark sinks rapidly also, but if such shoots are exposed for a time to sunlight their respiration is considerably increased (1, 2). So also, if the carbohydrate content of etiolated leaves, shoots, or seedlings is increased by an immersion of the parts in sugar solutions, respiration is greatly stimulated, although Palladine attributes the increased respiration partly to the formation of active proteins produced under conditions of favorable carbohydrate nutrition (5, 8, 9).

Since the sugar content of sweet potatoes (*Ipomoea batatas*) changes greatly in storage, it appeared not unlikely in view of the foregoing facts that their respiratory activity would show corresponding changes at different seasons. The experiments described in the following pages were performed in order to ascertain whether any such correlation exists between the seasonal changes in the sugar content of sweet potatoes and their respiratory activity, and incidentally to determine if possible whether the monosaccharids or the disaccharids of the sweet potato furnish the chief material for respiration. The roots were taken from the lots stored for experimental purposes under the conditions described by the writers in a former paper (4). The details are given in connection with the descriptions of the individual experiments. The respiration

¹ Reference is made by number to "Literature cited," p. 517.

experiments were all carried out at 30° C. This temperature was chosen in order to study the respiration of the sweet potatoes under conditions similar to those to which the freshly dug roots are subjected during the curing process, which consists essentially in keeping them at a temperature in the neighborhood of 30° C. for about 10 days.

EXPERIMENTAL METHODS

The methods employed in the experiments require but little description. The sweet potatoes were placed in a large receptacle in an ordinary water-jacketed incubator, which was kept at a temperature of 30° C. A current of air having the same temperature and freed from carbon dioxid was drawn through the receptacle at the rate of 40 to 50 liters per hour. The carbon dioxid of respiration was collected in approximately one-half normal potassium-hydroxid solution, whose titre for pure potassium hydroxid had been determined. The absorption was effected by means of Reiset flasks. At the end of every 24-hour period the carbon dioxid in the Reiset flask was precipitated by means of an excess of barium chlorid, and the residual potassium hydroxid was determined by titration with normal or half-normal hydrochloric acid.

About 2 to 3 kgm. of sweet potatoes were used in each experiment. At the beginning of the experiment the sugar content was determined in a collateral sample of 3 to 4 kgm. from the same lot. At the end of each experiment all the sweet potatoes which had been used for that experiment were ground and sampled for determinations of sugar and moisture. The figures giving the sugar determinations are averages of five samples from each lot. The directly reducing sugar was calculated as glucose. The soluble carbohydrates yielding reducing sugar after inversion were calculated as cane sugar, which is the most abundant disaccharid present in the sweet potato. Jersey Big Stem sweet potatoes were used in all the experiments.

EXPERIMENTAL DATA

The results of all the experiments are collected in Table I. The percentages of total sugar (as glucose), cane sugar, and reducing sugar (as glucose) in the collateral sample taken at the beginning of each experiment, and in the experimental sweet potatoes at the end of the experiment, are given at the head of the table. These figures were in each case calculated for sweet potatoes of the water content of the collateral sample—i. e., the assumed original water content of the experimental sweet potatoes. The carbon-dioxid output is given in milligrams per kilogram per hour for each day. In the calculation the loss of weight of the sweet potatoes during the experiment was taken into consideration and was distributed uniformly over the period. At

the end of Table I is given the gain or loss of reducing sugar, calculated from the analytical data, and the glucose equivalent of the total carbon dioxid generated during each experiment, as actually determined. The percentages of reducing sugar in the sweet potatoes at the end of each experiment, without correction for changes in water content, were as follows: First experiment, 1.24 per cent; second, 1.22 per cent; third, 1.39 per cent; fourth, 0.91 per cent; fifth, 0.71 per cent; sixth, 0.67 per cent; seventh, 0.69 per cent. The experiments themselves will be described individually.

TABLE I.—*Composition and carbon-dioxid output of sweet potatoes at different times of the year*

Item.	Period.	Experiment 1, Oct. 21 to Nov. 5.	Experiment 2, Nov. 7 to Nov. 17.	Experiment 3, Dec. 9 to Dec. 19.	Experiment 4, Jan. 4 to Jan. 15.	Experiment 5, Mar. 26 to Apr. 5.	Experiment 6, Apr. 16 to Apr. 26.	Experiment 7, June 1 to June 11.
Total sugar (as glucose), per cent.	At beginning of experiment.	2.62	5.80	8.99	6.22	7.03	7.41	7.30
	At end of experiment.	5.38	5.08	8.42	5.82	7.45	7.40	7.20
Cane sugar, per cent.	At beginning of experiment.	1.60	3.41	6.58	4.63	5.82	6.17	6.08
	At end of experiment.	3.95	3.72	6.74	4.71	6.42	6.41	6.21
Reducing sugar, per cent.	At beginning of experiment.	.94	2.21	2.06	1.35	.90	.92	.90
	At end of experiment.	1.23	1.18	1.35	.87	.70	.67	.68
Daily rate of carbon-dioxid output, mgm. per kgm. per hour.	1st day.....	27.7	73.9	138.2	49.1	50.9	46.6	47.5
	2d day.....	24.9	82.1	144.9	56.0	44.9	49.4	42.4
	3d day.....	36.5	70.9	116.4	54.1	47.0	48.4	43.4
	4th day.....	35.7	60.0	101.8	48.4	46.8	46.4	46.2
	5th day.....	37.1	51.8	92.9	48.2	47.5	43.3	42.9
	6th day.....	31.8	45.9	90.4	44.0	47.0	42.0	43.1
	7th day.....	41.7	40.4	84.9	42.6	46.1	42.1	40.7
	8th day.....	34.3	39.1	83.3	40.7	46.5	39.3	42.2
	9th day.....	31.6	34.8	76.7	39.8	44.9	40.8	41.0
	10th day.....	29.8	32.8	79.9	38.9	41.1	39.3	44.1
	11th day.....	28.8	41.4
	12th day.....	28.5
	13th day.....	24.9
	14th day.....	31.7
	15th day.....	29.9
Increment in reducing sugar calculated from the analytical data, gm.	9.77	31.69	17.35	10.03	3.40	5.14	2.50
Loss of reducing sugar equivalent to the carbon dioxid evolved, gm.	27.45	25.85	35.18	15.80	11.73	13.41	7.33

EXPERIMENT 1.—In this experiment 3,576.5 gm. of sweet potatoes were used. These were dug on October 20. The experiment was begun on the following day and continued until November 5. During that period the cane-sugar content rose from 1.60 to 3.95 per cent and the invert-sugar content from 0.94 to 1.23 per cent. The respiration rose somewhat during the first half of the period and then fell to a nearly uniform rate of approximately 28 mgm. per kilogram per hour. The rise at first, which was observed in nearly all the other experiments also, may in part be attributed to the rise of the temperature of

the sweet potatoes when they were put into the incubator. Although there is a marked increase in both cane sugar and reducing sugar in the sweet potatoes, there is no evident general rise in the respiratory activity corresponding to the increase in the sugar content. During the course of the experiment the equivalent of 27.45 gm. of glucose was given off by the sweet potatoes as carbon dioxid, yet during this period 9.77 gm. of reducing sugar accumulated in them. The loss of weight of the sweet potatoes was 77 gm.

EXPERIMENT 2.—The sweet potatoes used in the second experiment were of the same lot as those of the first, but they had stood in the laboratory at a temperature of about 20° C. until November 7. The weight of the roots used for the experiment was 3,029.8 gm. The loss of weight was 138.8 gm. The percentage of cane sugar rose slightly, but the reducing sugar fell from 2.21 to 1.18 per cent. The respiration was high at first and fell gradually, apparently with the decreasing percentage of reducing sugar. It is clear that if in this case the lowering of the respiratory activity is due to the decrease of sugar, the effect must be wholly attributed to the change in the invert-sugar content, since the cane sugar, so far as may be judged from the analysis of the collateral sample, remained stationary or even rose slightly. The changes in the quantity of reducing sugar in these sweet potatoes are of special interest, for here the quantity of reducing sugar lost, according to calculations based on the analytical data, is greater than that lost through respiration as calculated from the quantity of carbon dioxid evolved. It seems, therefore, that a portion of the reducing sugar was used for other processes than respiration, possibly for the production of cane sugar.

EXPERIMENT 3.—The sweet potatoes used in the third experiment had been subjected to the regular curing process and had thereafter been kept in cold storage at a temperature of 6° to 7° C. from November 8 to December 9. The roots used in the experiment weighed 2,207.2 gm., and their loss of weight was 184.2 gm. As a result of the exposure to low temperature, the sugar content of these sweet potatoes was higher than of those used in any of the other experiments. The respiration of these chilled roots was also very high, but sank rapidly toward the end of the experiment. The quantity of reducing sugar equivalent to the carbon dioxid evolved in respiration was greater than the apparent decrease calculated from the analytical data.

EXPERIMENTS 4, 5, 6, AND 7.—The remaining experiments all present a certain uniformity and may be described together. The sweet potatoes used in these experiments were cured in the usual manner and were thereafter stored at a temperature of 12° to 15° C., until the dates on which they were used. The weights of the sweet potatoes used in the different experiments were 1,984, 1,577.5, 1,898.5, and 1,054.5 gm., respectively. The corresponding losses were 143, 56.5, 59.3, and 40.8

gm. The sugar content of these lots was remarkably uniform. Only the lot used in the fourth experiment was lower in cane sugar and higher in reducing sugar than the rest. In spite of this difference, the respiration in all cases was practically the same, beginning in the neighborhood of 50 mgm. per kilogram per hour and falling to about 40 mgm. toward the end of the experiments. In all cases the glucose equivalent of the carbon dioxid generated was higher than the loss of reducing sugar calculated from the analytical data.

DISCUSSION OF RESULTS

A comparison of the sugar content of the sweet potatoes in the different experiments with the respiration of the roots shows that no general correlation is evident between the total sugar content and the respiratory activity. It is true, indeed, that the roots having the highest sugar content (third experiment) also had the highest respiration, but these sweet potatoes had been subjected to low temperature for a month, and it is likely that such treatment induces other changes than those indicated by the carbohydrate transformations, for sweet potatoes thus treated become subject to the attacks of certain fungi which ordinarily do not readily invade the tissues. Moreover, it appears from experiments of Palladine (10) that, with a plentiful supply of carbohydrates present, plant organs which have been exposed for a time to low temperature respire more energetically when brought into a high temperature than those which have been continually kept at the higher temperature. Furthermore, the carbon-dioxid production in the third experiment fell off rapidly until it was no greater than that at the beginning of the second experiment, but the total sugar content of the sweet potatoes in the third experiment remained at all times much higher than that of the roots in the second experiment. The other experiments also show no correlation between the total sugar content of the sweet potato and the respiratory activity. Thus, the roots in the second experiment were low in total sugar, but had a high respiration, while those in the fifth, sixth, and seventh experiments had a comparatively high sugar content and low respiration. It is possible that irregularities in the size and shape of the sweet potatoes might account for differences in respiratory activity, but these sources of error were avoided as far as possible by the selection of fairly uniform roots. It is therefore unlikely that great differences in respiratory activity can be attributed to these factors.

While there appears to be no evident correlation between the total sugar content and the respiratory activity, the case is different when the reducing sugar alone is considered. Here there is evidence of a general parallelism, which, however, is easily obscured by other factors. This correlation is perhaps most clearly brought out by the gradual fall of the respiration, with the disappearance of the reducing sugar in the indi-

vidual experiments. The first experiment, however, is in marked contrast to the others in this respect, for, although the sugar content of these sweet potatoes rose from 0.94 to 1.23 per cent, there was no corresponding rise in the respiration. The parallelism between the respiration and the sugar content is less marked when the different experiments are compared. Thus, the roots in the second experiment contained approximately the same percentage of reducing sugar as those in the third, yet the respiration was much lower in the second. This fact, as has been pointed out, may probably be ascribed to the treatment to which the sweet potatoes had been subjected before the experiment. It is evident on the whole that the respiratory activity of the sweet potatoes is as greatly influenced by seasonal changes and environmental factors to which they have been exposed as by the sugar content. It is clear, of course, that with the exhaustion of the carbohydrates immediately utilized in respiration, the rate of respiration will fall, as in the case of seedlings grown continually in the dark, but it seems that an increase of the available carbohydrate supply does not necessarily entail a continued increase in the respiratory activity. That there is sufficient sugar present in sweet potatoes, as well as in plant organs generally, to support a more active respiration than usually takes place, is shown by the increased respiration as a result of wounding. Table II gives the carbon-dioxid output per kilogram per hour of two lots of sweet potatoes for a short period before and after they were split lengthwise.

TABLE II.—Carbon-dioxid output in milligrams per kilogram per hour of two lots of sweet potatoes for a short period before and after being split lengthwise

Before roots were split.			After roots were split.		
Days.	Output of carbon dioxid at 5° C.	Output of carbon dioxid at 30° C.	Days.	Output of carbon dioxid at 5° C.	Output of carbon dioxid at 30° C.
	Mgm.	Mgm.		Mgm.	Mgm.
1.....	4.4	42.7	7.....	9.3	60.0
2.....	4.1	39.2	8.....	6.9	50.8
3.....	4.7	36.3	9.....	7.2	52.7
4.....	5.4	35.4	10.....	7.2	70.7(?)
5.....	5.7	32.8	11.....	7.4	56.4
6.....	5.6	29.8	12.....	7.3	54.5
			13.....	7.6	52.5

The great increase in respiration after the sixth day, when the roots were split, shows that there was sufficient sugar present to support a more energetic respiration than that which took place in the whole roots, but that other limiting factors than the sugar supply determined the rate of respiration.

In the consideration of the question of the relative availability of the monosaccharids and the disaccharids as sources of material for respira-

tion, a certain allowance should perhaps be made for the nonconformity of samples, since the sugar content of the sweet potatoes at the beginning of each experiment was necessarily determined in collateral samples. Nevertheless, two facts appear evident. During the course of the experiments there was no diminution, but, on the contrary, an increase, in the quantity of cane sugar present in the sweet potatoes, while there was a marked decrease in the reducing sugar in all the experiments except the first.

The rise in the cane-sugar content of the sweet potatoes is most marked in the first experiment, but in this case the rapid change is simply an example of the generally observed manifestation that the sugar content of sweet potatoes is low while they are in the ground and rises rapidly immediately after they have been dug. In all the other experiments, although the increase is small (from 0.08 to 0.6 per cent), the differences all point in one direction. It seems clear, therefore, that there was at any rate no decrease in the cane-sugar content of the sweet potatoes during the course of the experiments.

This fact indicates that at 30° C. the cane sugar is reformed as rapidly as it is used for respiration or that it does not function in the respiratory processes, at least while other carbohydrates are present in abundance. Which of these possibilities occurs can not with certainty be determined from the data. A number of relative facts, however, seem to point to a rather high degree of stability of the cane sugar in the sweet potato, in so far as the processes of respiration are concerned. It has been found as the result of many analyses that at low temperatures (5° C.) there is an extensive accumulation of cane sugar in the sweet potato and that this increase of sugar takes place at the expense of the starch, which disappears correspondingly. At higher temperatures (15° to 20° C.) the accumulation of cane sugar is much less extensive and, in fact, does not proceed beyond a certain maximum, which, during the season's storage, is reached in March or early April. After the period of sugar formation the starch content of the sweet potatoes remains fairly constant, for the quantity of starch which disappears in respiration compared with the quantity used in the formation of sugar is so small that in view of individual differences among sweet potatoes and the errors of manipulation it has not been possible to determine the changes in starch content in connection with respiration in experiments carried on for short periods of time.

These facts seem to indicate that at higher temperatures the production of cane sugar is depressed. We should therefore expect that if sweet potatoes which have been stored at 15° to 20° C. until the cane-sugar content has attained an equilibrium (March to April) are subjected to a temperature of 30°, the production of cane sugar would be still further retarded or even inhibited. At the same time the rate of respiration is accelerated.

If no more cane sugar is formed and its utilization is hastened, we should expect a reduction in the quantity of cane sugar, at least in the experiments at the end of the season, if that substance is used in respiration. Such a reduction, however, occurs neither at the end of the season nor at any other time. It appears not unlikely, therefore, that the cane sugar in the sweet potato is relatively stable, with respect to the respiratory processes.

Although there was no diminution of cane sugar in the sweet potatoes used in these experiments, there was a marked decrease in the reducing sugar in all cases except the first. The first experiment, in which freshly dug roots were used, is exceptional for the reason mentioned above. It shows that in freshly dug roots the processes of sugar formation are so rapid that even at 30° C. sugar is formed faster than it is used in respiration. In this instance an amount of carbon dioxid equivalent to 27.45 gm. of glucose was evolved during the experiment, and in addition to this there was an increment of 9.77 gm. of reducing sugar, as calculated from the percentages present in the sweet potatoes at the beginning and at the end of the experiment. In all the other experiments there was a decrease of reducing sugar—i. e., the quantity of reducing sugar which had accumulated while the sweet potatoes were stored at low temperatures was diminished when the roots were subsequently exposed to a higher temperature. It is reasonable to infer that the sugar was utilized in respiration, but it will be observed that in all but the first and second experiments the loss of reducing sugar calculated from the percentages at the beginning and at the end of the experiments accounts only for a portion of the sugar equivalent to the quantity of carbon dioxid evolved. The deficiency is no doubt made up by the transformation of starch, for, as Deleano (3) found in the case of grape leaves cut from the vines, the starch functions readily in the respiratory processes. In the sweet potato the starch appears to be even more readily available than the cane sugar. In the second experiment, where the invert-sugar content was high at the beginning of the experiment, a synthesis of other carbohydrates may perhaps be assumed.

CONCLUSIONS

The experiments described in this paper seem to indicate that there is no general correlation between the total sugar content of the sweet potato and its respiratory activity. A simultaneous decrease in the reducing-sugar content and the respiratory activity of given lots of roots indicates a correlation between reducing-sugar content and respiration, but seasonal changes and environmental conditions to which the sweet potatoes have been previously subjected tend to obscure any such correlation in different lots. Experiments with wounded roots indicate that the sugar content is not the limiting factor in the respiration of the sweet potato. The reducing sugars are the immediate source of respira-

tory material. The cane sugar is relatively stable in the sweet potato, and when once formed it does not appear to be readily utilized in the process of respiration, while starch and other carbohydrates are present in abundance.

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CHERRY AND HAWTHORN SAWFLY LEAF MINER

By P. J. PARROTT, *Entomologist*, and B. B. FULTON, *Assistant Entomologist*, New York Agricultural Experiment Station, Geneva, N. Y.

INTRODUCTION

The existence in the State of New York of a leaf miner attacking cherry (*Prunus* spp.) foliage was brought to the attention of the Experiment Station by the receipt of affected foliage during the latter part of June, 1910. An examination of the orchard from which the material had been collected showed that more or less of the leaves on nearly all of the trees of a variety known as English Morello had shriveled and died, while here and there were others with well-defined light-colored areas or blisters, revealing a loss of chlorophyll. Siftings of earth from beneath the trees showed that the causal agent was the larva of a species of sawfly. A number of these were carried through successive stages of development to the following year, when adults were obtained. Some specimens were forwarded to Dr. A. D. MacGillivray, formerly of Cornell University, who reported that the insect represented a new species, the type of a new genus, and should be recorded as *Profenusa collaris*. The information was also given that the creature had been reared from the hawthorn (*Crataegus* spp.).

HOST PLANTS OF SAWFLY LEAF MINER

According to present knowledge, the host plants of the sawfly leaf miner are the cherry and the hawthorn. Of the cherries, it has so far largely confined its attacks to the English Morello variety. It is not commonly observed with the Montmorency or Early Richmond, which would indicate that its presence on these varieties is accidental and occurs when they are grown in proximity to the English Morello. The susceptibility of one fruit and the apparent unattractiveness or resistance to the insect of the other fruits is a curious fact, since all are cultivated varieties of the same cherry, *Prunus cerasus*, and plantings of each kind, growing side by side, may be frequently observed in this State. The two sorts, Montmorency and English Morello, represent groups of cherries which vary more or less in both tree and fruit but have a constant difference only in a single character—the juice in the fruits of one is colorless; in the other it is red. This sharp discrimination on the part of the sawfly leaf miner seems all the more anomalous when considered in the light of its extreme partiality to the foliage of certain hawthorns which are only remotely related to the cherry.

In its attacks on hawthorns the leaf miner tunnels the foliage in the same manner as that of the cherry. During the course of our studies it has been very evident that the pest is more destructive to certain species of *Crataegus* than it is to the English Morello cherry. As has been rarely observed in the case of the latter plant, one may find as many as five larvæ mining a single leaf. With hawthorns having a relatively small and narrow leaf, as *C. geneseensis*, there may be an entire destruction of the pulpy tissue, in which event all that remains of the affected leaf is the epidermis, which dries up and ultimately falls to the ground. At the height of an attack, which occurs when the larvæ are reaching maturity, hawthorns which are much infested take on a brownish cast and appear as if struck by a blight or swept by fire. In decorative plantings the destructive work of the insect may assume such a character that the attractiveness of certain species of hawthorns as ornamental shrubs is seriously marred.

About Geneva the sawfly leaf miner is most common in the foliage of an unidentified hawthorn belonging to the *Medioximæ* group, while such species as *C. pedicellata* and *C. punctata*, growing in the immediate vicinity of the former, have so far shown little or no injury and are generally exempt from attack. Dr. C. S. Sargent, Director of the Arnold Arboretum, writes that the insect has become established in the plantings of *Crataegus* spp. and that it is especially destructive to hawthorns of the *crus-galli* group and to *C. nitida*, *C. rotundifolia*, *C. pruinosa*, and other species. Similar conditions exist at the New York Botanical Garden and, as elsewhere, certain species of *Crataegus* are quite badly infested, while a few species have so far been free from attack.

In the public parks at Rochester, N. Y., notably Genesee Park, the insect has in recent years become a serious pest. Hawthorns representing a wide range of species and grown in extensive numbers feature prominently in certain landscape plantings. In these the sawfly leaf miner has become established, and its destructiveness may be readily observed during May and June. Some haws have been seriously affected, while others have been exempt from injury. Here, again, various hawthorns of the *crus-galli* group have proved to be very susceptible to the pest, and certain species of other groups have shown considerable injury.

DISTRIBUTION OF SAWFLY LEAF MINER.

As a cherry pest the sawfly leaf miner is definitely known to occur in injurious numbers in orchards of English Morello cherry about Geneva in western New York and about Germantown, which is located in the Hudson Valley. It has been reported to the Station as occurring about Schenectady, but the statement of its presence in that locality has not been verified. In view of its occurrence in two communities which are widely separated, it would seem reasonable to suppose that the pest exists in

other localities where sour cherries are extensively grown. However, a careful survey by the orchard and nursery inspectors of the Department of Agriculture in all of the leading fruit-growing counties of the State has failed to find any evidences of the work of the insect except in the foregoing localities. A study of available literature indicates that the insect is not known to occur as a cherry pest outside the State of New York.

As a depredator of hawthorns the sawfly leaf miner has a wider range of distribution. It is known, as already indicated, as a serious pest of hawthorns growing about Boston, Mass., and it is common on various species of *Crataegus* growing in the vicinity of New York City, Rochester, Ithaca, Geneva, and Skaneateles, all of which are located in the State of New York.

APPEARANCE OF THE INJURY

As implied by its common name, the insect is a leaf-mining species and its work is very characteristic. The injury is first indicated by a small, thin, sinuous channel which finally swells out into a large blister-like area of a light-brown color, resembling that of dead leaf tissues. The attack by the larva of the sawfly leaf miner begins on the edge of the leaf toward the stem and continues along one side toward the leaf apex, the tunnel increasing in dimensions with the growth in size and the progress of the insect. Upon reaching the tip of the leaf the grub reverses its course and works backward toward the stem, consuming the remainder of the pulpy tissues between the main rib and the margin of the leaf. As a result, the parenchyma, or soft cellular tissue, is eaten, leaving the epidermis, which turns brown and forms a large blister. These blisters are very conspicuous on the upper surfaces of the leaves. Oftentimes the whole leaf is mined, but usually with most of the foliage only from one-quarter to one-half of the whole area of a leaf is destroyed. (Pl. LI, fig. 1.) Only the leaves that first unfold are subject to attack, and during some seasons hardly any of these escape the insect's depredations. The principal damage occurs during the last week of May and the early part of June, or about one month before the harvesting of the fruit. With the disappearance of the larvæ the leaves most seriously affected shrivel, die, and finally drop to the ground, causing defoliation, which varies in importance according to the extent of infestation and the influence of seasonal conditions on the rate of growth.

The actual effect of the work of the insect upon the crop is not easily measured and during most years is perhaps not of serious extent. However, as previously indicated, the destructive power of the pest is mainly exercised on the leaves that unfold with the bursting of the buds. In years of slight precipitation and when new growth is of small extent and of slow development the plant is dependent on such foliage as it carries at the time, and any extended injury to it must result in a set-

back, with correspondingly ill effects on the maturing crop of fruit. In years when the production of new growth is more rapid the damage caused by the sawfly leaf miner is of much less importance, as the large leaf surface under the circumstances is sufficient for the needs of the plant, and the loss of affected foliage does not result in an important reduction in leaf area.

The hawthorns are more subject to severe attacks than the cherry, and during some seasons plants may be observed on which there is hardly a leaf that does not show injury. Notwithstanding the partiality of the sawfly leaf miner for this plant, hawthorns seem able to withstand considerable destruction of foliage without marked external evidences of the weakening of the tree. As shown in Plate LI, figure 2, the attractiveness of the plants as ornamental shrubs may be seriously marred.

DESCRIPTION OF LIFE STAGES OF SAWFLY LEAF MINER

EGG

The egg is elliptical in shape, but is not entirely symmetrical in its outline, as one side shows a greater curvature than the other. It is, when removed from surrounding plant tissues, circular in cross section, but in its normal position in the leaf structure it is much flattened, owing to pressure. The chorion is a thin, white, shining, flexible membrane. The measurements of eggs when not compressed are: Length, 0.5 to 0.7 mm.; diameter, 0.28 to 0.36 mm.

LARVA

To determine the number of instars, the mines were carefully examined for all insect remains, when the head molts were collected and measured as to width. The body remnants from some of the molts in first larval instars were occasionally missing, having probably been eaten, but in very few cases were the head structures not in good condition for examination. The width of the head is fairly constant for the first larval instar, but in the more advanced stages there is considerable variation. On the basis of head measurements it appears that the larva normally molts five times in its mine. It finally enters the ground and molts again in transforming to a pupa.

The first five instars have the same general form and differ one from the other principally in size. The body is broadest at the first and second thoracic segments and gradually tapers toward the rear. The thoracic legs are short and conical and are composed of five segments, which include the thick basal and the small hooked terminal structures. All the abdominal segments except the last bear short rounded prolegs on the ventral side. The head is horizontal in the early stages, but slopes downward slightly in later instars. It is broad and flat, rounded on the sides, and obtuse in front. On the dorsal side it bears four longitudinal sutures. The outer pair run back from the ends of the clypeus and divide the head into three almost equal sections. The inner pair extend halfway across the middle section, dividing it into three equal areas. The eyes are wanting. The antennæ are very short and are apparently composed of three segments. The maxillary palpi are large and protrude from beneath the head. The labial palpi are very small. The mandibles are short and thick, deeply hollowed on the inner side, and do not protrude beyond the end of the broadly notched labrum.

The technical description of each of the larval stages follows:

FIRST INSTAR.—Body translucent, white, shining; only slightly wrinkled, and with a green streak, due to alimentary tract, showing plainly in the abdominal segments. Prolegs appear as only slight elevations.

Head is slightly brownish, being of dark color on the outer and posterior edges; mouth parts are reddish brown. The ventral side of the first thoracic segment has a pair of brownish gray marks, shaped roughly like a T, with the cross bar running longitudinally and the perpendicular reaching outward to a point just in front of the leg. A semicircular line of the same color occurs in front of the anus and is interrupted on the median line.

Newly hatched larvæ are about 1.2 mm. in length, and after feeding, the body grows, reaching a length of 2.3 mm. Width of head, 0.36 to 0.42 mm.; average, 0.39 mm.

SECOND INSTAR.—All markings of body are more extensive than in preceding stage. Dorsal side with some specimens has a broad, faint, brownish gray, transverse band on the first thoracic and two spots on the second thoracic segment. The pair of marks on ventral side of first thoracic segment are shaped more like inverted V's, and between them there is a large longitudinal band. The second and third segments have median oval spots. Each proleg is marked by a narrow crescent on the anterior side. A semicircular mark on the last segment extends over half a circle and is not interrupted on the median line.

Length, 2.6 to 3 mm. Width of head, 0.48 to 0.55 mm.; average, 0.52 mm.

THIRD INSTAR.—All markings are the same as in preceding stage, but are much fainter. Prolegs are more prominent; those on the first and penultimate abdominal segments are small.

Length, 3.2 to 4.3 mm. Width of head, 0.63 to 0.73 mm.; average, 0.67 mm.

FOURTH INSTAR.—The characteristic markings in preceding stages practically disappear in this instar. A ring of several rows of minute papillæ surrounds the anus. These probably exist in the earlier instars and escape detection because of their small size.

Length, 4.5 to 7.2 mm. Width of head, 0.8 to 0.9 mm.; average, 0.85 mm.

FIFTH INSTAR.—This is similar to fourth instar. There are no distinct color markings.

Length, 6.5 to 7.5 mm. Width of head, 0.92 to 1.07 mm.; average, 1 mm.

SIXTH INSTAR.—The body does not differ from that of preceding stage. The head assumes a vertical position. The four sutures on the dorsal side are very faint. The clypeus and labrum are shorter than in fifth instar. The mandibles protrude prominently and do not meet at the ends. The labium and maxillæ project from beneath the head to beyond the tips of the mandibles.

Length is same as in fifth instar or may be a trifle shorter. Width of head, 0.90 to 1.05 mm.; average, 1 mm.

PUPA

Until color of adult begins to show, the pupa is white in all portions except the eyes, which are reddish. Length about 5 mm.

ADULT

"Body [of female] black, with the clypeus, labrum, malar space, the mandibles, the first segment of the antennæ, the tegulæ, a narrow margin to the pronotum, and the legs, for the most part, whitish. The prothorax, except the parts named, the cephalic part of the mesopleuræ, and the pectus, rufous; the posterior femora more or less shaded with fuscous; the head smooth with antennal furrows interrupted on the middle of the face; the furrows surrounding the postocellar area deep and distinct, the vertical furrows not reaching the occiput; the median ocellus placed on a flat depression; a pit above the antennal socket; the median fovea minute but dis-

tinged; the clypeus truncate; the first and second antennal segments subequal, the third segment subequal to one and two together and longer than four; the saw-guides with the dorsal and ventral margins converging and the apex bluntly pointed; the male differs in having the rufous part of the thorax inclined to whitish and extending over the entire pleuræ, the venter of the abdomen and a broad band on the lateral part of the dorsal aspect, broader behind, sometimes fused on the meson, whitish; the posterior femora not fuscous. Length 3 to 4 mm."¹

LIFE HISTORY AND HABITS OF SAWFLY LEAF MINER

EMERGENCE OF ADULTS

From puparia obtained on April 18, 1913, by sifting earth from beneath cherry trees, two male and seven female sawfly leaf miners made their appearance during a period extending from April 28 to May 2. On May 6 six males and six females were obtained in a cherry orchard, and only one of the flies was obtained in cages intended to trap the insects as they emerged from the ground. On May 7 five males and seven females were caught in breeding cages, and at this date the insects were present in large numbers on the trees. The insects continued to appear in the cages, a few each day, until May 19, which for 1913 was the latest date for the emergence of the flies for that year. Observations for several seasons show that the flies make their appearance when the first leaf clusters are unfolding and the cluster buds are beginning to open.

EARLY HABITS

At the time of their emergence from the ground the sawfly leaf miners are fully colored and are very active creatures. They are apparently very susceptible to temperature conditions. If disturbed on cold days, they drop suddenly from the foliage, attempting to fly while in midair. Failing in this effort, they drop to the ground and crawl to some elevated object, on which they renew their attempts to seek flight.

They copulate within less than a day after their appearance from the soil. In this act the male approaches the female backward, so that the tips of their abdomens come in contact while their heads are opposed to each other. Then the male reaches back with the hind legs and grasps the female over the back of her body, placing at the same time the tip of his abdomen under that of the female and inserting the penis under the flap at the base of the ovipositor. The outer flaps of the male genitalia are pressed closely against the under side of the female's body. The whole process is a matter of one to three minutes. One pair contained in an observation jar copulated three times within a space of half an hour.

OVIPOSITION

The females are apparently ready to oviposit soon after they make their escape from the ground. One specimen was dissected about 17 hours after its appearance, and in the ovaries and oviducts there were

¹ MacGillivray, A. D. New genera and species of sawflies. *In* *Canad. Ent.*, v. 46, no. 10, p. 364-365. 1914.

counted 15 fully developed eggs. Another that had been out for two days began to deposit eggs immediately when cherry leaves were introduced into its cage. In the orchard eggs were first found during the year 1913 on May 7; in that season adults were first observed on May 6, although the insects may have been present on the trees for a day or two before and escaped detection. During the first days of the oviposition period one or sometimes two leaves in a cluster may show the presence of eggs. The females seem to manifest a preference for leaves which are first to appear and which are partly folded. The process of oviposition requires only about a minute. Details of this operation proved difficult to determine because of the extreme shyness of the females, which fly quickly on the approach of any object.

The lower surface of the egg lies in contact with the lower epidermis, which has been cut free from the other tissues of the leaf so as to form a small blister-like cavity or pocket. The egg is usually within 1 or 2 mm. from the edge of the leaf; rarely on the extreme edge or more than 3 mm. from the margin. On the upper side at the edge of the cavity there is usually a stoma, through which the ovipositor is probably thrust. An examination of 91 eggs at random shows that they are more often deposited near the base of the leaf than the tip. About 70 per cent of the eggs were in the area of the leaf from one-eighth to one-third the distance from the base, 20 per cent near the middle, and about 10 per cent occurred in the portion of the leaf toward the tip. From 1 to 5 eggs were observed on a single leaf, and the average for all observations was 2.3 eggs per leaf.

HATCHING AND LARVAL ACTIVITIES

During 1913 young larvæ were first observed on May 24 as the trees were coming into full bloom, but judging from the sizes of some of the mines it was evident that a few eggs had hatched one or two days earlier. By May 27 the hatching period was practically completed. In the field it proved difficult to determine the period of incubation, but eggs deposited on cherry leaves in the insectary hatched in eight days from date of oviposition. Under normal conditions incubation would probably extend over a larger number of days.

Upon hatching, the young larva works its way through the tissue of the leaf until it reaches the upper epidermis. It usually mines toward the distal end of the leaf, generally keeping close to the edge and feeding with the ventral side in contact with the upper epidermis. When the tip of the leaf has been reached the creature reverses its course, proceeding along the area adjoining the midrib; or if there is no interference by another larva it may cross over the main rib and tunnel back along the edge of the opposite half of the leaf.

The mine, as viewed from above, during its first stages of development is rather dark brown in color, which is accounted for in part by frass along the edges of the roof of the tunnel. As the affected area increases

in size, especially in its breadth, the mine becomes light brown, while the edges incline to a darker shade. Observed from beneath, the only visible indication of the initial activities of the insect is a small oval spot, which marks the original cavity constructed by the adult for the reception of the egg, and this contains in addition to the shriveled egg membrane accumulations of frass from the early feeding operations of the larva. Later, the underside of the tunnel also becomes brown, with the exposed epidermis wrinkled, but, in general, the destructive work of the insect is not so apparent on the lower as on the upper surface of the leaf.

There is a fairly definite relationship between the size of the mine and the age of the larva with respect to the different instars. In general, mines under 5 mm. long and 2 mm. at their greatest width contain larvæ in the first instar; mines that are 5 by 2 mm. to 12 by 4 mm. contain larvæ in the second instar; mines that are 8 by 5 mm. to 8 by 6 mm. contain larvæ of the third instar; mines that are 18 by 6 mm. to 28 by 8 mm. contain larvæ of the fourth instar; and mines of greater dimensions than the foregoing are occupied by larvæ of the fifth instar.

PUPATION

Upon reaching maturity the larvæ make a hole in the tissues forming the mine, usually the upper epidermis, which forms the roof. From the opening they make their escape to the edge of the leaf, when they drop to the ground. During 1912 the larvæ began to leave the foliage on June 7, and by June 10 it was estimated that 50 per cent of the insects had abandoned their mines. On June 18 it was difficult to find a specimen on the tree, while June 22 was the latest date that any of the insects were seen on the leaves. Upon reaching the ground they bury themselves several inches deep in the soil and construct an earthen cell. The cocoon, which is oval in shape, consists of particles of earth glued together and lined with a cement which renders it impervious to water and strong enough to resist considerable pressure without crushing. The insect passes the winter in the larval stage. However, the pupa begins to form in the fall. Specimens obtained during October showed the developing compound eyes and ocelli, while of examples secured the following April the adult characters of the head could be plainly seen through the skin, and their bodies were decidedly humped. One of these specimens which was kept in a cool room transformed to a pupa on or before April 23. Others obtained from an orchard on May 2, 1913, were all in the pupal stage, and one female pupa was partly colored.

NATURAL ENEMIES OF SAWFLY LEAF MINER¹

A common enemy of the sawfly leaf miner is the chalcidid *Trichogramma minutum* Riley, which is an egg parasite. During the five years that

¹ Through the courtesy of Dr. L. O. Howard, the identifications of the parasites were made by Messrs. A. A. Girault and A. S. Rohwer, of the United States Bureau of Entomology.

Profenusa collaris has been under observation, *T. minutum* has twice made its appearance in conspicuous numbers in infested cherry orchards, in 1912 and in 1915. During the former year the larger percentage of the eggs of the leaf miner were attacked, and on some trees it was difficult to find an egg-bearing leaf which had not been visited by the parasite. In 1915 parasitism ranged from about 40 to 90 per cent on individual trees. Taking all trees into consideration, of the eggs deposited by the insect a larger percentage of them certainly failed to hatch than hatched, and for this mortality *T. minutum* appeared to be largely responsible.

The parasite was reared from both cherry and hawthorn foliage. The majority of the eggs of the leaf miner that were dissected contained a single parasite, and in only a few instances were twin larvæ or pupæ observed. On June 2, 1915, the parasites were all in the larval state, but on June 5, when the larvæ of *P. collaris* were beginning to abandon their mines in the foliage, about 50 per cent of the parasites were in the pupal state. By June 7 they had nearly all transformed to pupæ, and on June 9 the first adult appeared. During succeeding days the chalcids appeared in large numbers, and the last specimen to make its appearance emerged on June 14. While the parasite was abundant about Geneva during this year, it was relatively quite scarce on plantings of *Crataegus* spp. at Rochester.

Besides the foregoing parasite there has been reared from *P. collaris* an ichneumon which proved to be a new species and has been listed by Rohwer¹ as "*Pezoporus tenthredinarum*." Apparently there is associated with this ichneumon an undescribed tryphonine, but owing to the small numbers collected it is impossible to make any definite statement at this time as to its status as a parasite of the sawfly leaf miner.

METHODS OF CONTROL

REMOVAL OF AFFECTED LEAVES

Of the operations systematically practiced, one that will probably prove most effective and economical in controlling the sawfly leaf miner is the picking of affected leaves. This species is peculiarly susceptible to this kind of repressive method, since there is only one brood of larvæ to attack the foliage, and oviposition extends over only a short period. The effect is that hatching of eggs and maturing of larvæ are, practically speaking, almost simultaneous for all of the creatures, and their activities during their injurious stages are therefore restricted to a relatively short period. By careful timing it is possible at a single picking to collect practically all of the larvæ by removing the affected leaves, which should then be burned to destroy the insects therein. The removal and destruction of all mined leaves, coupled with another practice—the destruc-

¹ Rohwer, S. A. Descriptions of new species of Hymenoptera. In Proc. U. S. Nat. Mus., v. 49, p. 216. 1915.

tion of wild hawthorns in the immediate vicinity of the cherry orchard—should leave few opportunities for the pest to develop to injurious numbers.

FUMIGATION WITH HYDROCYANIC-ACID GAS

Of the various measures employing insecticides tested by this station to protect cherry foliage from the work of the leaf miner, fumigation with hydrocyanic-acid gas alone was effective. Most cherry growers in New York are not equipped with suitable apparatus to undertake this means of affording protection to their trees, and fumigation should only be undertaken as an extreme measure and in an experimental way under expert direction.

CULTIVATION

Cultivation, if done with care and at the proper time, is destructive to many insects with subterranean habits. Species especially that undergo pupal development in the ground are not only peculiarly sensitive to disturbances of the soil, but plowing and cultivation, besides breaking up the cells of hibernating larvæ, exert another detrimental influence, exposing the helpless insects to insectivorous birds and other foes. Since it is the normal habit of the larvæ of this sawfly leaf miner to live in earthen cells for the greater portion of the life cycle of the species, such practices as fall or early spring plowing or cultivation are to be recommended from an entomological standpoint. These measures, fortunately, are standard operations which are invariably practiced by the most successful cherry growers.

DESTRUCTION OF UNCULTIVATED HOST PLANTS

The fact that the sawfly leaf miner is very partial to hawthorns, especially of the group *C. crus-galli*, and breeds most abundantly on them, suggests the desirability of destroying these plants when they exist in the immediate vicinity of a cherry orchard. The value of this operation is not known; but until there is more knowledge of the breeding habits of the pest the removal of wild plants along roadsides and hedgerows that are attractive to the insect for purposes of propagation would appear advisable as a precautionary measure.

SPRAYING OF HAWTHORNS

For the protection of hawthorns in decorative plantings, spraying seems to be preferred to any of the preceding measures. The insecticide which has given the most satisfactory results is composed of 1 pint of nicotine solution (40 per cent) to 100 gallons of water to which are added 4 pounds of soap. In making the treatment the liquid should be used in liberal amounts and applied with rather high pressures at the time when the insects first begin to mine the foliage.

PLATE LI

Fig. 1.—Leaves of English Morello cherry, showing injury by the sawfly leaf miner.

Fig. 2.—Leaves of hawthorn, showing injury by the sawfly leaf miner.



VARIATIONS IN MINERAL COMPOSITION OF SAP, LEAVES, AND STEMS OF THE WILD-GRAPE VINE AND SUGAR-MAPLE TREE

By O. M. SHEDD,¹

Chemist, Kentucky Agricultural Experiment Station

INTRODUCTION

In a previous publication Kastle and the writer (9)² have shown the relation existing between the mineral components of the sap of the wild-grape vine (*Vitis cordifolia*) and those contained in the young leaves and stems at a certain period in its growth during the same year. At that time these writers stated that they did not know whether these relations would hold true throughout the growing season, and they purposed to continue the investigation so as to include the sap and other materials from different portions of this vine and other plants.

Since our former publication, the writer has found in the literature at hand that considerable work has been done by Chandler (1), Harris and Gortner (8), Dixon and others (2, 3, 4, 5, 6, 7) on the physiochemical properties of certain saps or plant juices, but, so far as we have been able to find, no work has been done on the mineral composition of the sap or on the changes occurring therein which might have any bearing on the above-mentioned investigation.

EXPERIMENTS WITH WILD-GRAPE VINE

With this idea in view, the writer has during the last three years (1912-1914) collected samples of the sap from the vine employed in the former work, in order to determine (1) whether the mineral composition of this sap varies at the same time in different parts of the vine, (2) whether it varies during a single season at a certain point, and (3) whether it varies during different years. The analyses are of interest, inasmuch as they show large differences in the composition of the sap, depending on the time and place of collection. The results are given in Tables I to XI and are expressed in percentage by weight, except where otherwise stated. The mineral components of the original sample have been calculated from the amounts found in the ash, except the chlorin, which was determined in the fresh sap. The sulphur-trioxid content of the original substance is probably low, since more or less sulphur is lost in ashing organic materials.

¹ The author desires to express his gratitude to Dr. J. H. Kastle, Director of the Kentucky Experiment Station, for his helpful advice during the progress of this investigation.

² Reference is made by number to "Literature cited," p. 541-542.

In order to understand more fully the different tabulations, a brief description of each sample follows.

Nos. 285, 812, and 852 were collected in April of 1912, 1913, and 1914, respectively, from the cut end of the same main branch about 20 feet from the root of the vine and just after the sap flow commenced.

No. 853 was collected in April, 1914, from the cut end of another main branch about 4 feet from the root of the vine and just after the sap flow commenced. This sample was taken at the same time as No. 852.

No. 854 was collected in April, 1914, from the same point as No. 852, but seven days later and just before the sap flow ceased.

No. 900 was collected in April, 1915, from the cut end of one of the main branches about 20 feet from the root of the vine and just after the sap flow commenced. This was a different branch from that from which No. 285 was taken, because no sap exuded from the old branch, and it seemed to have been greatly weakened by the annual loss.

No. 901 was collected in April, 1915, from several of the small branches or shoots which were of the previous year's growth and just after the sap flow commenced. This sample was taken at the same time as No. 900 and from 10 shoots which were located several feet from the main branches.

Nos. 902, 904, and 906 were collected for three successive days from 9 a. m. to 5 p. m., beginning on April 29, 1915, four days after and from the same point as No. 900.

Nos. 903, 905, and 907 were collected for three successive nights from 5 p. m. to 9 a. m., beginning on April 29, 1915, and from the same point as No. 900.

The variation in the percentage composition of the fresh sap and the ash of samples 852, 853, 900, and 901 are given in Tables I and II.

TABLE I.—*Variation in percentage composition of fresh sap collected at the same time from different points on the wild-grape vine*¹

Constituent.	Sample No. 852.	Sample No. 853.	Sample No. 900.	Sample No. 901.	Ratio between—	
					Nos. 852 and 853.	Nos. 900 and 901.
Water at 100° C.....	99.8279	99.8538	99.8183	99.8431	1 : 1.00	1 : 1.00
Organic matter.....	.1435	.1112	.1305	.1068	1 : .77	1 : .82
Silica (SiO ₂).....	.0001	.0001	.0003	.0017	1 : 1.00	1 : 5.67
Ferric and aluminic oxids (Fe ₂ O ₃ +Al ₂ O ₃).....	.0001	.0001	.0001	.0004	1 : 1.00	1 : 4.00
Calcium oxid (CaO).....	.0160	.0155	.0234	.0268	1 : .97	1 : 1.15
Magnesium oxid (MgO).....	.0024	.0025	.0041	.0062	1 : 1.04	1 : 1.51
Sodium oxid (Na ₂ O).....	.0012	.0012	.0010	.0011	1 : 1.00	1 : 1.10
Potassium oxid (K ₂ O).....	.0050	.0112	.0167	.0074	1 : 2.24	1 : .44
Phosphorus pentoxid (P ₂ O ₅).....	.0015	.0026	.0030	.0030	1 : 1.73	1 : 1.00
Sulphur trioxid (SO ₃).....	.0019	.0017	.0025	.0033	1 : .89	1 : 1.32
Chlorin.....	.0004	.0001	.0001	.0002	1 : .25	1 : 2.00
Total.....	100.0000	100.0000	100.0000	100.0000
d ²⁵ / ₂₅	1.0009	1.0008	1.00082	1.00027	1 : 1.00	1 : 1.00
Nitrogen as nitrates.....	.0013	.0024	.00004	.00001	1 : 1.85	1 : .25
Crude ash.....	.0384	.0477	.0700	.0662	1 : 1.24	1 : .95

¹ Nos. 852 and 853 were collected in 1914; Nos. 900 and 901 in 1915.

TABLE II.—Percentage composition of ash of the samples in Table I

Constituent.	Sample No. 852.	Sample No. 853.	Sample No. 900.	Sample No. 901.	Ratio between—	
					Nos. 852 and 853.	Nos. 900 and 901.
Silica (SiO_2).....	0.339	0.231	0.485	2.505	1 : 0.68	1 : 5.16
Ferric and aluminic oxids ($\text{Fe}_2\text{O}_3 + \text{Al}_2\text{O}_3$).....	.261	.210	.143	.574	1 : .80	1 : 4.01
Calcium oxid (CaO).....	41.628	32.535	33.432	40.386	1 : .78	1 : 1.21
Magnesium oxid (MgO).....	6.364	5.296	5.828	9.424	1 : .83	1 : 1.62
Sodium oxid (Na_2O).....	3.234	2.522	1.483	1.633	1 : .78	1 : 1.10
Potassium oxid (K_2O).....	13.146	23.465	23.787	11.103	1 : 1.78	1 : .47
Phosphorus pentoxid (P_2O_5).....	3.860	5.380	4.349	4.543	1 : 1.39	1 : 1.04
Sulphur trioxid (SO_3).....	5.008	3.531	3.502	5.052	1 : .71	1 : 1.42
Carbon dioxide, not determined						
Total.....	73.840	73.170	73.069	75.220		

From an examination of Table I it is apparent that the water, calcium, and sodium content of the sap are fairly constant when collected at two different points at the same time during the same year, while the silica, iron, aluminum, potassium, phosphorus, and chlorin are the large variable constituents, depending on the time and point of collection. The organic matter is higher in the sap taken at a point on the main branch about 20 feet from the root than it is on the same branch closer to the ground or on the new branches. The silica, iron, aluminum, calcium, magnesium, and sulphur, however, are higher in the sap in the new branches. These facts agree with the writer's previous findings, which show that the minerals accumulate in the leaves. As the grapevine puts forth leaves every year only on the parts of more recent growth, the above results are what one would naturally expect when considered in connection with the former work.

Another interesting point is that certain constituents—namely, silica, iron, aluminum, magnesium, and phosphorus—may be about the same in the sap when collected from two different points at the same time during a given year, but vary widely when compared the following season.

A further point of interest is that while the ratio of calcium oxid to magnesium oxid is fairly constant in each sap of Table I, that of the potassium oxid to sodium oxid is variable, as shown in Table III.

TABLE III.—Comparison of the ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in sap of the wild grape collected at the same time from different points on the vine

Sample No.	Ratio of calcium oxid to magnesium oxid.	Ratio of potassium oxid to sodium oxid.
852.....	6.7 : 1	4.2 : 1
853.....	6.2 : 1	9.3 : 1
900.....	5.7 : 1	16.7 : 1
901.....	4.3 : 1	6.7 : 1

TABLE IV.—*Variation in percentage composition of fresh sap collected at the same point on the wild-grape vine at different times during the same season*¹

Constituent.	Sample No. 852.	Sample No. 854.	Sample No. 900.	Sample No. 902. ^b	Ratio between—	
					Nos. 852 and 854.	Nos. 900 and 902.
Water at 100° C.....	99.8279	99.7545	99.8183	99.7026	1 : 1.00	1 : 1.00
Organic matter.....	.1435	.1821	.1305	.2208	1 : 1.27	1 : 1.69
Silica (SiO ₂).....	.0001	.0003	.0003	.0007	1 : 3.00	1 : 2.33
Ferric and aluminic oxids (Fe ₂ O ₃ +Al ₂ O ₃)..	.0001	.0001	.0001	.0003	1 : 1.00	1 : 3.00
Calcium oxid (CaO).....	.0160	.0221	.0234	.0277	1 : 1.38	1 : 1.18
Magnesium oxid (MgO).....	.0024	.0036	.0041	.0047	1 : 1.50	1 : 1.15
Sodium oxid (Na ₂ O).....	.0012	.0013	.0010	.0011	1 : 1.08	1 : 1.10
Potassium oxid (K ₂ O).....	.0050	.0277	.0167	.0316	1 : 5.54	1 : 1.89
Phosphorus pentoxid (P ₂ O ₅).....	.0015	.0045	.0030	.0069	1 : 3.00	1 : 2.30
Sulphur trioxid (SO ₃).....	.0019	.0037	.0025	.0036	1 : 1.95	1 : 1.44
Chlorin.....	.0004	.0001	.0001	1 : .25
Total.....	100.0000	100.0000	100.0000	100.0000
d ^{25°}	1.0009	1.0007	1.00082	1 : 1.00
Nitrogen as nitrates.....	.0013	.0028	.00004	1 : 2.15
Crude ash.....	.0384	.0863	.0700	.1012	1 : 2.25	1 : 1.45

¹ Nos. 852 and 854 were collected in 1914; Nos. 900 and 902, in 1915.^b Composition by volume, but this does not appreciably affect the percentage by weight.TABLE V.—*Percentage composition of ash of samples in Table IV*

Constituent.	Sample No. 852.	Sample No. 854.	Sample No. 900.	Sample No. 902.	Ratio between—	
					Nos. 852 and 854.	Nos. 900 and 902.
Silica (SiO ₂).....	0.339	0.371	0.485	0.678	1 : 1.09	1 : 1.40
Ferric and aluminic oxids (Fe ₂ O ₃ +Al ₂ O ₃).....	.261	.093	.143	.254	1 : .36	1 : 1.78
Calcium oxid (CaO).....	41.628	25.627	33.432	27.386	1 : .62	1 : .82
Magnesium oxid (MgO).....	6.364	4.225	5.828	4.602	1 : .66	1 : .79
Sodium oxid (Na ₂ O).....	3.234	1.486	1.483	1.078	1 : .46	1 : .73
Potassium oxid (K ₂ O).....	13.146	32.080	23.787	31.198	1 : 2.44	1 : 1.31
Phosphorus pentoxid (P ₂ O ₅).....	3.860	5.269	4.349	4.771	1 : 1.37	1 : 1.56
Sulphur trioxid (SO ₃).....	5.008	4.271	3.562	3.564	1 : .85	1 : 1.00
Carbon dioxide, not determined.....
Total.....	73.840	73.422	73.069	73.531

In Table IV it appears that in both years there is a concentration of practically all the minerals in the sap at the end of the sap flow, or when new leaves develop, compared with the beginning. The ratio of increase of some of the minerals—namely, silica, iron, aluminum, potassium, phosphorus, and sulphur—in one or both years is much greater than the remainder. There is also a wide variation in the percentages of ash in the different samples, which partly accounts for some of these differences (Table V). Furthermore, an examination of the ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid shows that the former remains fairly constant, while the latter is variable and demon-

strates the large amount of potassium oxid in the sap at the end of the sap flow compared with the beginning, since the sodium oxid is fairly constant during both years. See Table VI.

TABLE VI.—*Comparison of the ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in sap of wild grape taken from the same point on the vine at different times during the same season*

Sample No.	Ratio of calcium oxid to magnesium oxid.	Ratio of potassium oxid to sodium oxid.
852.....	6.7 : 1	4.2 : 1
854.....	6.1 : 1	21.3 : 1
900.....	5.7 : 1	16.7 : 1
902.....	5.9 : 1	28.7 : 1

An examination of the minimum and maximum percentages of the minerals in the sap collected at the same point during four successive years and just after the sap flow commenced shows the largest variations which have been found (Table VII). The constituents vary in order of magnitude as follows: Potassium, chlorin, iron, aluminum, silica, phosphorus, sulphur, magnesium, sodium, and calcium. Again there is a wide variation in the ash content of the different samples (Table VIII).

TABLE VII.—*Variation in percentage composition of fresh sap collected at the same point on the wild-grape vine at the beginning of the sap flow during four successive years*

Constituent.	Sample No. 285.	Sample No. 812.	Sample No. 852.	Sample No. 900.	Ratio between minimum and maximum.
Water at 100° C.....	99.6340	99.8665	99.8279	99.8183	1 : 1.00
Organic matter.....	.2782	.0917	.1435	.1305	1 : 3.03
Silica (SiO ₂).....	.0005	.0005	.0001	.0003	1 : 5.00
Ferric and aluminic oxids (Fe ₂ O ₃ + Al ₂ O ₃).....	.0006	.0002	.0001	.0001	1 : 6.00
Calcium oxid (CaO).....	.0220	.0206	.0160	.0234	1 : 1.46
Magnesium oxid (MgO).....	.0044	.0043	.0024	.0041	1 : 1.83
Sodium oxid (Na ₂ O).....	.0017	.0010	.0012	.0010	1 : 1.70
Potassium oxid (K ₂ O).....	.0468	.0112	.0050	.0167	1 : 9.36
Phosphorus pentoxid (P ₂ O ₅).....	.0058	.0017	.0015	.0030	1 : 3.87
Sulphur trioxid (SO ₃).....	.0052	.0016	.0019	.0025	1 : 3.25
Chlorin.....	.0008	.0001	.0004	.0001	1 : 8.00
Total.....	100.0000	100.0000	100.0000	100.0000
d ₂₅ ^{25°}	1.0035	1.00067	1.0009	1.00082	1 : 1.00
Nitrogen as nitrates.....	.0075	.00048	.0013	.00004	1 : 187.50
Crude ash.....	.1130	.0570	.0384	.07000	1 : 2.94

TABLE VIII.—Percentage composition of ash of samples in Table VII

Constituent.	Sample No. 285.	Sample No. 812.	Sample No. 852.	Sample No. 900.	Ratio between minimum and maximum.
Silica (SiO ₂).....	0.405	0.809	0.339	0.485	1 : 2.39
Ferric and aluminic oxids (Fe ₂ O ₃ +Al ₂ O ₃).....	.540	.387	.261	.143	1 : 3.78
Calcium oxid (CaO).....	19.490	36.070	41.628	33.432	1 : 2.14
Magnesium oxid (MgO).....	3.900	7.594	6.364	5.828	1 : 1.95
Sodium oxid (Na ₂ O).....	1.500	2.742	3.234	1.483	1 : 2.16
Potassium oxid (K ₂ O).....	41.380	19.617	13.146	23.787	1 : 3.15
Phosphorus pentoxid (P ₂ O ₅).....	5.090	3.059	3.860	4.349	1 : 1.66
Sulphur trioxid (SO ₃).....	4.590	2.742	5.008	3.562	1 : 1.83
Carbon dioxid, not determined.....					
Total.....	76.895	73.020	73.840	73.069

As stated before, Nos. 285, 812, and 852 were collected from the same branch, whereas No. 900 was taken an equal distance from the root on another branch, as the former was so greatly weakened that no sap exuded from it at the proper time, although new growth came on it later, showing that it was not dead. If a comparison now be made of Nos. 285, 812, and 852, it will be found that there has been a marked reduction in practically all of the mineral substances in the sap in the two succeeding years compared with the first, and, moreover, this was very sharp in some constituents in the second and, in others, in the third year. Furthermore, it will be noticed that among those which show a decided decrease in the second year are potassium and phosphorus, both of which are included among the chief essential plant-food elements.

According to the different analyses of the sap, potassium is among the high mineral constituents, and as this element has shown the largest loss, this may account for the weakened condition of the branch.

The ratios of calcium oxid to magnesium oxid and of potassium oxid to sodium oxid in the various samples of Table VII are as given in Table IX.

TABLE IX.—Comparison of ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in sap of wild grape from the same point on the vine at the beginning of the sap flow during four successive years

Sample No.	Ratio of calcium oxid to magnesium oxid.	Ratio of potassium oxid to sodium oxid.
285.....	5.0 : 1	27.5 : 1
812.....	4.8 : 1	7.0 : 1
852.....	6.7 : 1	4.2 : 1
900.....	5.7 : 1	16.7 : 1

Table IX shows that the ratio of calcium oxid to magnesium oxid is fairly constant in the different samples, while the wide variation in the potassium oxid and sodium oxid from 27.5 in 1912 to 4.2 in 1914 would indicate that these figures were obtained from the sap of different plants rather than from that of the same vine at different times.

TABLE X.—*Variation in percentage composition¹ of fresh sap of wild grape collected for three successive days and nights²*

Constituent.	Sample No. 902.	Sample No. 903.	Sample No. 904.	Sample No. 905.	Sample No. 906.	Sample No. 907.	Ratio between minimum and maximum.
Water at 100° C.	99.7026	99.7354	99.7436	99.7473	99.7592	99.7469	1 : 1.00
Organic matter.	.2208	.1971	.1766	.1892	.1732	.1874	1 : 1.27
Silica (SiO ₂)	.0007	.0006	.0008	.0007	.0005	.0007	1 : 1.60
Ferric and aluminic oxids (Fe ₂ O ₃ +Al ₂ O ₃)	.0003	.0002	.0005	.0001	.0005	.0001	1 : 5.00
Calcium oxid (CaO)	.0277	.0248	.0248	.0245	.0228	.0233	1 : 1.21
Magnesium oxid (MgO)	.0047	.0042	.0089	.0049	.0070	.0045	1 : 2.12
Sodium oxid (Na ₂ O)	.0011	.0008	.0039	.0008	.0041	.0014	1 : 5.13
Potassium oxid (K ₂ O)	.0316	.0279	.0296	.0239	.0245	.0254	1 : 1.32
Phosphorus pentoxid (P ₂ O ₅)	.0069	.0060	.0077	.0056	.0054	.0067	1 : 1.43
Sulphur trioxid (SO ₃)	.0036	.0030	.0036	.0030	.0028	.0036	1 : 1.29
Chlorin, not determined							
Total	100.0000	100.0000	100.0000	100.0000	100.0000	100.0000	
Crude ash	.1012	.0916	.0925	.0843	.0780	.0839	1 : 1.30

¹ By volume.

² Nos. 902, 904, and 906 were collected on successive days; Nos. 903, 905, and 907 were collected on successive nights.

TABLE XI.—*Percentage composition of ash of samples in Table X*

Constituent.	Sample No. 902.	Sample No. 903.	Sample No. 904.	Sample No. 905.	Sample No. 906.	Sample No. 907.	Ratio between minimum and maximum.
Silica (SiO ₂)	0.678	0.691	0.884	0.804	0.641	0.788	1 : 1.38
Ferric and aluminic oxids (Fe ₂ O ₃ +Al ₂ O ₃)	.254	.267	.590	.079	.641	.110	1 : 8.11
Calcium oxid (CaO)	27.386	27.068	26.820	29.034	29.167	27.796	1 : 1.09
Magnesium oxid (MgO)	4.602	4.621	9.607	5.840	8.939	5.366	1 : 2.09
Sodium oxid (Na ₂ O)	1.078	.823	4.220	1.006	5.269	1.672	1 : 6.40
Potassium oxid (K ₂ O)	31.198	30.478	31.986	28.277	31.430	30.232	1 : 1.13
Phosphorus pentoxid (P ₂ O ₅)	6.771	6.544	8.271	6.604	6.950	7.999	1 : 1.26
Sulphur trioxid (SO ₃)	3.564	3.261	3.841	3.524	3.628	4.327	1 : 1.33
Carbon dioxide, not determined							
Total	75.531	73.753	86.219	75.168	86.665	78.290	

Referring to the results in Table X, it will be seen that there is a considerable variation occurring daily in the mineral composition of the sap and that, as a rule, most of its constituents are present in larger amounts during the day, while, on the other hand, its composition is more constant at night (Table XI).

As there was such a wide variation in short periods of time in the composition of the sap of this vine, it was thought desirable to collect further samples of the young leaves and stems in order to determine if this would hold true in regard to these parts. Accordingly, in June, 1915, or two months after the sap was first collected, and every two weeks thereafter for six weeks, samples of the succulent young stems and leaves representing the same stage of growth were taken. Therefore, the results are somewhat comparable with each other and with those formerly obtained, since the earlier samples were taken in a similar manner in Nos. 908 and 909. The consecutive analyses are given in Tables XII to XV along with those of Nos. 627 and 628 of 1912.

TABLE XII.—*Variation in percentage composition of young green leaves of wild-grape vine in the same and in different years*

Constituent.	Sample No. 627.	Sample No. 908.	Sample No. 910.	Sample No. 912.	Sample No. 914.	Ratio between minimum and maximum.
Water at 100° C.	75.4700	75.1200	71.1975	73.1525	72.6015	1:1.06
Organic matter.	22.8500	23.3181	26.8851	25.3750	25.9097	1:1.18
Silica (SiO ₂).1372	.0514	.0642	.0291	.0537	1:4.71
Ferric and aluminic oxids (Fe ₂ O ₃ +Al ₂ O ₃)..	.0214	.0197	.0394	.0117	.0165	1:3.37
Calcium oxid (CaO)...	.7200	.5478	.7585	.5515	.4427	1:1.71
Magnesium oxid (MgO)..	.1337	.1112	.1333	.1004	.1078	1:1.33
Sodium oxid (Na ₂ O)...	.0356	.0167	.0214	.0236	.0287	1:2.13
Potassium oxid (K ₂ O)...	.3427	.5619	.5943	.5076	.5752	1:1.73
Phosphorus pentoxid (P ₂ O ₅).....	.2260	.2104	.2020	.1891	.1992	1:1.20
Sulphur trioxid (SO ₃)...	.0634	.0428	.1043	.0595	.0650	1:2.44
Total.....	100.0000	100.0000	100.0000	100.0000	100.0000
Crude ash.....	2.3300	2.0140	2.4595	1.9530	1.8790	1:1.31

TABLE XIII.—*Percentage composition of ash of samples in Table XII*

Constituent.	Sample No. 627.	Sample No. 908.	Sample No. 910.	Sample No. 912.	Sample No. 914.	Ratio between minimum and maximum.
Silica (SiO ₂).	5.890	2.550	2.610	1.490	2.860	1:3.95
Ferric and aluminic oxids (Fe ₂ O ₃ +Al ₂ O ₃)..	.920	.980	1.600	.600	.880	1:2.67
Calcium oxid (CaO)	30.900	27.200	30.840	28.240	23.560	1:1.31
Magnesium oxid (MgO)..	5.740	5.520	5.419	5.143	5.737	1:1.12
Sodium oxid (Na ₂ O)....	1.530	.827	.870	1.209	1.527	1:1.85
Potassium oxid (K ₂ O)..	14.710	27.899	24.163	25.992	30.613	1:2.08
Phosphorus pentoxid (P ₂ O ₅).....	9.700	10.447	8.215	9.682	10.600	1:1.29
Sulphur trioxid (SO ₃)...	2.720	2.127	4.239	3.046	3.457	1:1.99
Carbon dioxid, not determined.....
Total.....	72.110	77.550	77.956	75.402	79.234

TABLE XIV.—*Variation in percentage composition of young green stems of wild-grape vine in the same and in different years*

Constituent.	Sample No. 628.	Sample No. 909.	Sample No. 911.	Sample No. 913.	Sample No. 915.	Ratio between minimum and maximum.
Water at 100° C.	79.2500	84.2750	81.9385	83.3210	82.6645	1 : 1.06
Organic matter.	20.0437	14.8654	17.0893	15.7397	16.4453	1 : 1.35
Silica (SiO ₂)0041	.0048	.0069	.0037	.0040	1 : 1.86
Ferric and aluminic oxids (Fe ₂ O ₃ +Al ₂ O ₃)0003	.0051	.0038	.0041	.0032	1 : 17.00
Calcium oxid (CaO)1114	.1558	.2244	.2488	.1792	1 : 2.23
Magnesium oxid (MgO)0346	.0539	.0642	.0567	.0557	1 : 1.86
Sodium oxid (Na ₂ O)0171	.0078	.0133	.0158	.0093	1 : 2.19
Potassium oxid (K ₂ O)3883	.4813	.5154	.4846	.5098	1 : 1.33
Phosphorus pentoxid (P ₂ O ₅)1277	.1055	.1078	.1010	.1009	1 : 1.27
Sulphur trioxid (SO ₃)0228	.0454	.0364	.0246	.0281	1 : 1.99
Total	100.0000	100.0000	100.0000	100.0000	100.0000
Crude ash	1.0200	1.1490	1.2810	1.3790	1.2175	1 : 1.35

TABLE XV.—*Percentage composition of ash of samples in Table XIV*

Constituent.	Sample No. 628.	Sample No. 909.	Sample No. 911.	Sample No. 913.	Sample No. 915.	Ratio between minimum and maximum.
Silica (SiO ₂)	0.400	0.420	0.540	0.270	0.330	1 : 2.00
Ferric and aluminic oxids (Fe ₂ O ₃ +Al ₂ O ₃)030	.440	.300	.300	.260	1 : 14.67
Calcium oxid (CaO)	10.920	13.560	17.520	18.040	14.720	1 : 1.74
Magnesium oxid (MgO)	3.390	4.604	5.013	4.115	4.578	1 : 1.48
Sodium oxid (Na ₂ O)	1.680	.679	1.039	1.145	.764	1 : 2.47
Potassium oxid (K ₂ O)	38.070	41.892	40.241	35.140	41.876	1 : 1.19
Phosphorus pentoxid (P ₂ O ₅)	12.520	9.184	8.419	7.322	8.291	1 : 1.71
Sulphur trioxid (SO ₃)	2.240	3.951	2.840	1.784	2.305	1 : 2.21
Carbon dioxide, not determined
Total	69.250	74.820	75.912	68.116	73.124

In Tables XII and XIV it will be found that the ratio between the lowest and the highest result obtained for each of the other mineral constituents in the several samples of leaves and stems is more constant than it is for the silica, iron, and aluminum. It will further be found that the results obtained this year on the different samples corroborate, except in one instance, those of 1912 in showing that there is a concentration of all the constituents in the leaf compared with the stem, and the exception is that whereas the potassium content of the stem in 1912 was greater than in the leaf, this year (1915) it was less in every case.

In the leaf the silica, sodium, magnesium, and phosphorus are uniformly lower than in 1912 and the organic matter and potassium are higher, while the other constituents vary both above and below the former results. In the stem, however, the organic matter, sodium, and phosphorus are lower than formerly and the iron, aluminum, calcium, magnesium, potassium, and sulphur are higher, while the silica is variable.

Another interesting point is that most of the results show that the mineral constituents are lower in the leaves of this year (1915) than formerly, while in the stem they are higher.

The ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in the leaf and stem below show, as did those of the sap, that the former is more constant than the latter (Table XVI).

TABLE XVI.—Comparison of ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in leaves and stems of young wild-grape vine in the same and in different years

Part and sample No.	Ratio of calcium oxid to magnesium oxid.	Ratio of potassium oxid to sodium oxid.
Leaf:		
627	5. 4 : 1	9. 6 : 1
908	4. 9 : 1	33. 6 : 1
910	5. 7 : 1	27. 8 : 1
912	5. 5 : 1	21. 5 : 1
914	4. 1 : 1	20. 0 : 1
Stem:		
628	3. 2 : 1	22. 7 : 1
909	2. 9 : 1	61. 7 : 1
911	3. 5 : 1	38. 8 : 1
913	4. 4 : 1	30. 7 : 1
915	3. 2 : 1	54. 8 : 1

EXPERIMENTS WITH SUGAR MAPLE

Having found such a wide variation in the composition of the sap of the wild-grape vine, it was thought that it might prove of further interest to compare the analyses of the sap of the same sugar-maple tree (*Acer saccharum*) collected during two successive years. Accordingly, early in 1913 and 1914, just after the sap began to rise, samples were collected at the same point on the tree, about 3 feet from the ground.

Also, for a further comparison, the sap was collected in 1913, just after the sap flow commenced, from a water-maple tree (*Acer saccharinum*) at a point about 10 feet from the ground.

The results are given in Tables XVII and XVIII.

TABLE XVII.—*Variation in composition of the sap of the water-maple and sugar-maple trees*

Constituent.	Water maple No. 744.	Sugar maple.		Ratio between Nos. 776 and 851.
		No. 776. ^a	No. 851. ^b	
Water at 100° C.....	98. 2035	98. 2953	98. 3227	1 : 1. 00
Organic matter.....	1. 7677	1. 6812	1. 6247	1 : . 97
Silica (SiO ₂).....	. 0013	. 0016	. 0011	1 : . 69
Ferric and aluminic oxids (Fe ₂ O ₃ +Al ₂ O ₃).....	. 0001	. 0001	. 0001	1 : 1. 00
Calcium oxid (CaO).....	. 0053	. 0097	. 0200	1 : 2. 06
Magnesium oxid (MgO).....	. 0009	. 0018	. 0026	1 : 1. 44
Sodium oxid (Na ₂ O).....	. 0020	. 0004	. 0009	1 : 2. 25
Potassium oxid (K ₂ O).....	. 0118	. 0084	. 0178	1 : 2. 12
Phosphorus pentoxid (P ₂ O ₅).....	. 0023	. 0007	. 0060	1 : 8. 57
Sulphur trioxid (SO ₃).....	. 0004	. 0002	. 0033	1 : 16. 50
Chlorin.....	. 0047	. 0006	. 0008	1 : 1. 33
Total.....	100. 0000	100. 0000	100. 0000
d_{25}^{25}	1. 0056	1. 0045	1. 0059	1 : 1. 00
Nitrogen as nitrates.....	. 0007
Crude ash.....	. 0296	. 0336	. 0678	1 : 2. 02

^a Collected in 1913 just after the sap flow commenced.^b Collected in 1914 just after the sap flow commenced, from same point on the same tree as No. 776.TABLE XVIII.—*Percentage composition of ash of samples in Table XVII*

Constituent.	Water maple No. 744.	Sugar maple.		Ratio be- tween Nos. 776 and 851.
		No. 776.	No. 851.	
Silica (SiO ₂).....	4. 444	4. 868	1. 701	1 : . 35
Ferric and aluminic oxids (Fe ₂ O ₃ +Al ₂ O ₃).....	. 506	. 237	. 220	1 : . 93
Calcium oxid (CaO).....	18. 003	28. 864	29. 561	1 : 1. 02
Magnesium oxid (MgO).....	2. 926	5. 399	3. 893	1 : . 72
Sodium oxid (Na ₂ O).....	6. 751	1. 241	1. 352	1 : 1. 09
Potassium oxid (K ₂ O).....	39. 945	24. 902	26. 211	1 : 1. 05
Phosphorus pentoxid (P ₂ O ₅).....	7. 651	2. 079	8. 881	1 : 4. 27
Sulphur trioxid (SO ₃).....	1. 238	. 518	4. 834	1 : 9. 33
Carbon dioxid, not determined.....
Total.....	81. 464	68. 108	76. 653

In Table XVII we find that the calcium, magnesium, sodium, potassium, phosphorus, and sulphur are much higher in the sugar-maple sap in 1914 than in 1913 and the silica is lower, while the water, organic matter, iron, and aluminum are about the same in both years. The largest varying constituents are sulphur and phosphorus.

Again, on comparing the sap of the sugar maple with that of the water maple, there are found large differences in the calcium, magnesium, sodium, potassium, phosphorus, sulphur, and chlorin, while the water, organic matter, silica, iron, and aluminum are about the same.

The large amount of sodium and chlorin in the sap of the water maple may be explained as due to the fact that this tree was located on a city lot and may have received sodium chlorid from the drainage, while the sugar maple was located in the country. On the other hand, the wild-grape vine was also on a city lot, but in a different locality, and its sap did not show a large chlorin content; still, this may have been due to the difference in the drainage of the two places.

The differences obtained in the mineral constituents of the several samples of sap can not be due altogether to the different moisture content of the soil, for the large variations in the ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid in the tables show that it is not a dilution of the sap by the soil water.

The moisture content of the soil at the time of the sap collection was not determined, and, of course, this would be influenced by several factors, such as temperature, rainfall, sunshine, and wind at that period. Taking into account the rainfall alone will not explain the differences obtained, as will be seen from Table XIX.

TABLE XIX.—*Rainfall in inches during four successive years*

Month.	1912.	1913.	1914.	1915.
January.....	1. 78	10. 35	2. 50	4. 38
February.....	2. 50	2. 61	3. 87	1. 12
March.....	4. 36	6. 04	2. 24	1. 49
April.....	6. 89	2. 41	2. 23	. 65

During the spring of this year (1915) there was less rainfall in this vicinity than for years, and there is no doubt that the moisture content of the soil at the time of the sap collection in 1915 was considerably lower than it was the three preceding years. If the results are to be explained from the dilution standpoint, then those of Nos. 285 and 900 in Table VII should be in harmony with what has just been stated, while, as a matter of fact, they are contradictory, except for one constituent.

The foregoing results show that the sap has a variable mineral composition which later on influences the structure of the growing parts, and this undoubtedly explains the differences in composition of the same and different varieties of plants.

SUMMARY

(1) There is considerable variation in the composition of the sap of the wild-grape vine when collected at the same time from two different points. This has been the case for two seasons.

(2) Large differences in the composition of this sap were found when it was collected at the same point on the vine at different times during the same season. The minerals in the sap are higher at the end of the

sap flow than at the beginning. This has also been proved for two seasons.

(3) The widest variations in the composition of this sap were found when it was collected at the same point on a main branch of the vine at the beginning of the sap flow during four successive years. The periodic loss of sap greatly weakened this branch, and there was also a steady decline in the mineral components of the sap taken from it, particularly potassium and phosphorus.

(4) There was found a considerable variation occurring daily in the composition of this sap. The mineral constituents were generally higher during the day and the sap had a more uniform composition during the night.

(5) The young leaves and stems of this vine at the same stage of growth were also found to vary considerably in composition during different years and also in the same season.

(6) The sap of the same sugar-maple tree was found to vary widely in composition when collected at the same point on the tree during two successive years just after the sap flow had commenced.

(7) The mineral composition of the sap of the water-maple tree was found to be different from that of the sugar maple.

(8) The ratios of calcium oxid to magnesium oxid and potassium oxid to sodium oxid, together with other factors, demonstrate that the differences in composition can not be altogether explained as being due to a dilution of the sap from the water in the soil.

(9) It has been shown that the sap has a variable mineral composition which influences the structure of the growing parts and undoubtedly explains the differences in composition of the same and different varieties of plants.

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CARBOHYDRATE TRANSFORMATIONS IN SWEET POTATOES

By HEINRICH HASSELBRING and LON A. HAWKINS, *Plant Physiologists, Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations, Bureau of Plant Industry*

INTRODUCTION

In a former paper¹ embodying a study of the general course of the carbohydrate transformations in sweet potatoes (*Ipomoea batatas*) during storage, certain data were presented indicating that the sugar content of sweet potatoes remains comparatively low while they are in the ground, but that immediately after the roots are harvested there is a transformation of starch into sugar which takes place more rapidly at that time than at subsequent periods. It was pointed out that this initial transformation seemed to be not greatly affected by temperature, but seemed rather to depend upon internal factors. It was suggested that possibly this initial change was associated with the cessation of the activity of the leaves. In view of the fact that this initial change appeared to be a phase of the carbohydrate metabolism of the sweet potato which was inaugurated only under certain conditions and which differed in some respects from subsequent changes, it seemed worth while to investigate this process more fully in order to make out something, if possible, as to its nature by a study of its progress at different temperatures.

EXPERIMENTATION IN CARBOHYDRATE TRANSFORMATION

PLAN OF EXPERIMENTS

The plan carried out in this work was, in general, to compare the carbohydrate transformations taking place in sweet potatoes during a period of 10 or 12 days immediately after they had been dug with the changes taking place during a second subsequent period of equal length. These experiments were carried out at temperatures of 30°, 15.5°, and 5° C.

¹ Hasselbring, Heinrich, and Hawkins, L. A. Physiological changes in sweet potatoes during storage. *In Jour. Agr. Research*, v. 3, no. 4, p. 331-342. 1915. Literature cited, p. 341-342.

The sweet potatoes used in the first series of experiments were dug on September 30, thoroughly washed, and were kept covered in the laboratory until the following day. In the further manipulation each potato was split lengthwise into two parts as nearly equal as possible. So far as could be determined the potatoes were cut longitudinally in a dorsiventral plane. One half, marked "a," was ground immediately, and samples were taken from the mash for the determination of moisture, sugar, and starch. The other half, marked "b," was stored. Six halves were stored at each temperature, the corresponding halves having been grated and sampled as described. The operation of preparing and sampling the halves for a set of experiments at the three temperatures required two days. Simultaneously with the halves a number of whole sweet potatoes were put into the constant-temperature chambers in which the experiments were conducted. At the end of 12 days the stored halves were taken out, grated, and sampled. After the completion of this operation, which required two days, the stored whole potatoes (which during this time had been subjected to the same conditions as the stored halves) were split lengthwise, like the first set, and one half was prepared for analysis. The other half was stored for another period of 12 days, after which it also was grated and analyzed. It will thus be noted that the difference in composition of the two halves of the first set of roots showed the change during the first period of 12 days immediately after the potatoes had been dug, while the difference in composition of the two halves of the second lot showed the change for a second period of 12 days immediately following the first period.

Although the time during which the sweet potatoes were exposed to the experimental conditions was essentially the same for the comparable lots, some unimportant differences necessarily crept in. Thus, for instance, it was impossible to prepare a complete set in a single day; therefore, one half of the potatoes used in the experiment were prepared one day and the other half the following day. Consequently, the one lot remained in the laboratory about a day longer than the other. Also, although the different groups were taken out of their respective chambers in the same order in which they were put in, no attention was paid to the order in which the individual potatoes were removed, since it was necessary to work as rapidly as possible. On this account it is likely that some halves remained in the chambers a few hours longer and others a few hours less than the assigned period, but it is obvious, considering the slowness of the changes that take place, that these discrepancies can have no effect on the general result.

The whole sweet potatoes stored simultaneously with the first set of halves also remained in the chambers two days longer than the halves, on account of the time required to grind and sample the stored halves; but this also is of no consequence, since the object of the experiments was

to compare the changes in the roots during the period immediately after they were dug with those during a subsequent period of the same length.

The second series of experiments was in all respects like the first except that the potatoes were dug on October 16 and placed in the experimental chambers on October 17 and 18. The length of time of storage was 12 days.

It was the object of the third series of experiments to determine the effect of removal of the vines on the initial carbohydrate changes in the sweet potato. The potatoes used in this series were, therefore, not dug until some time after the vines had been killed. The first frost, which killed the leaves but not the vines, occurred on October 22; a few days later, October 27, the vines were cut off close to the ground, so that from this time there would be no further transfer of materials from the vines to the roots. The potatoes were dug on November 6 and were thereafter treated as described for the other experiments, with the exception that the storage period was 10 days.

METHODS OF ANALYSIS

The methods of analysis were essentially the same as formerly described.¹ Only a few exceptions need be noted. The samples for moisture determinations were covered with 95 per cent alcohol as before, but the alcohol was evaporated in a drying oven at 50° C. Thereupon the samples were dried to their lowest weight in a vacuum oven in a slow current of air. This procedure gave clean, nearly white samples. For the starch determinations 10 gm. were weighed out and the whole sample, instead of an aliquot, was extracted, ground, and used for hydrolysis. The sugar samples were put into flasks, which were then nearly filled with 70 per cent alcohol, with the addition of a little calcium carbonate, and boiled for a minute or two. The starch samples were stored, without boiling, in 95 per cent alcohol.

GENERAL OBSERVATIONS ON THE EXPERIMENTS

In the experiments described halves of the same sweet potato were compared with each other, the one being analyzed immediately and the other at the end of a 10 to 12 day period of storage. Two questions immediately arise regarding this procedure, which was adopted because different sweet potatoes of the same variety differ much in composition: First, are the halves of the same potato alike in composition; and, second, do the cut potatoes behave in the same manner as whole potatoes in storage?

Müller-Thurgau² in his work on the common Irish potato found that there were only slight differences in the sugar content of the two halves

¹ Hasselbring, Heinrich, and Hawkins, L. A. Physiological changes in sweet potatoes during storage. *In Jour. Agr. Research*, v. 3, no. 4, p. 331-342. 1915. Literature cited, p. 341-342.

² Müller, Hermann, *Thurgau*. Ueber Zuckerrückbildung in Pflanzentheilen in Folge niedriger Temperatur. Ein Beitrag zur Kenntniss des Stoffwechsels der Pflanzen. *In Landw. Jahrb.*, Bd. 11, p. 751-828, pl. 26. 1882.

of potatoes cut lengthwise; it was therefore somewhat astonishing to find considerable differences in the two halves of sweet potatoes which were examined in July and August and which had been stored up to that time from the previous year. On this account further examinations were made of freshly dug sweet potatoes and of others of the same crop which had been stored in the chambers with the experimental sweet potatoes. The results of these examinations are given here.

On September 9 three freshly dug sweet potatoes were split dorsiventrally as nearly as could be judged. While one half was being prepared for analysis the other was kept wrapped in a damp cloth. From the mash of each grated half two samples were weighed out for sugar determinations, two for starch, and two for moisture. From each of the sugar samples and from each of the starch samples two determinations were made. The results calculated in percentages are given in Table I. The halves of the same potatoes are indicated by "a" and "b." The data afford an opportunity to estimate the error that is likely to occur in duplicate determinations in one sample, the error in sampling, and also the difference in composition of the halves of the same potato. The data show that the longitudinal halves of freshly dug sweet potatoes have very nearly the same composition and that errors due to method and technique are negligible.

TABLE I.—Percentage composition of halves of freshly dug sweet potatoes

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Cane sugar.	Starch.
1a.....	71.75	{ 0.66	2.27	21.00
		{ .66	2.23	20.99
1a.....	71.80	{ .68	2.20	21.00
		{ .68	2.25	20.95
1b.....	71.23	{ .79	2.17	21.42
		{ .78	2.21	21.47
1b.....	71.29	{ .75	2.21	21.43
		{ .77	2.25	21.53
2a.....	73.53	{ .87	2.01	19.58
		{ .85	2.00	19.47
2a.....	73.55	{ .90	1.95	19.69
		{ .90	1.93	19.55
2b.....	74.47	{ .94	1.95	18.32
		{ .95	2.03	18.44
2b.....	74.32	{ .98	2.00	18.28
		{ .96	2.01	18.16
3a.....	73.04	{ 1.13	1.74	19.59
		{ 1.15	1.68	19.78
3a.....	73.08	{ 1.16	1.68	19.60
		{ 1.16	1.60	19.58
3b.....	73.29	{	19.33
		{	19.39
3b.....	73.14	{ 1.29	1.71	19.21
		{ 1.32	1.66	19.25

To determine the effect of storage on the composition of different parts of the same sweet potato a number of other potatoes which had been stored for various lengths of time under different conditions were examined. One set of three potatoes was kept in the laboratory for four days. The other sets were stored for a month in the different chambers with the experimental potatoes.

In this case two samples were taken from each half, but the determinations were not made in duplicate for each sample. The results of these analyses are collected in Table II.

TABLE II.—Percentage composition of halves of sweet potatoes kept for various times under different conditions

KEPT IN LABORATORY FROM NOV. 6 TO NOV. 10

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Percentage of difference between halves.	Cane sugar.	Percentage of difference between halves.	Starch.	Percentage of difference between halves.
148a ..	74.64	1.21	9.9	3.01	2.6	16.36	1.4
148b ..	74.58	1.21		3.06		16.33	
149a ..	74.46	1.09	4.3	2.97	2.4	16.53	8.4
149b ..	74.52	1.09		2.94		16.61	
150a ..	76.48	1.41	16.4	3.51	.3	13.93	3.4
150b ..	76.48	1.40		3.52		14.09	
151a ..	75.49	1.36		3.43		15.22	
151b ..	75.48	1.33		3.43		15.16	
152a ..	76.32	.82		3.40		14.45	
152b ..	76.28	.83		3.36		14.68	
153a ..	76.72	.96		3.38		14.20	
153b ..	76.69	.96		3.40		13.93	

STORED IN 30° C. CHAMBER FROM NOV. 7 TO DEC. 5

110a ..	73.47	0.74	14.1	4.72	6.3	15.49	3.9
110b ..	73.44	.75		4.69		15.54	
111a ..	72.62	.64	6.3	4.97	2.2	16.21	3.1
111b ..	72.52	.64		5.03		16.04	
112a ..	72.29	.70		4.82		16.79	
112b ..	72.27	.72		4.78		16.57	
113a ..	72.06	.76		4.93		17.27	
113b ..	72.01	.75		4.88		17.14	

STORED IN 15.5° C. CHAMBER FROM OCT. 17 TO NOV. 11

68a ...	71.83	1.18	1.7	3.71	7.0	18.22	2.3
68b ...	71.84	1.16		3.73		18.21	
69a ...	70.99	1.16	4.2	3.98	1.3	18.71	6.7
69b ...	70.98	1.14		3.98		18.54	
70a ...	71.96	1.54		3.79		17.80	
70b ...	71.92	1.54		3.79		17.82	
71a ...	73.52	1.47		3.73		16.64	
71b ...	73.49	1.48		3.75		16.61	

TABLE II.—*Percentage composition of halves of sweet potatoes kept for various times under different conditions—Continued*

STORED IN 5° C. CHAMBER FROM OCT. 17 TO NOV. 11

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Percentage of difference between halves.	Cane sugar.	Percentage of difference between halves.	Starch.	Percentage of difference between halves.
89a ...	76. 30	2. 35	5. 5	4. 35	2. 5	12. 02	3. 9
	76. 32	2. 35		4. 38		12. 02	
89b ...	75. 79	2. 22		4. 48		12. 38	
	75. 71	2. 22	2. 5	4. 47	7. 2	12. 60	. 4
	75. 98	2. 61		3. 97		12. 44	
95a ...	75. 97	2. 61		3. 95		12. 40	
	76. 16	2. 57		3. 68		12. 48	
95b ...	76. 21	2. 52		3. 67		12. 45	

The results indicate that the longitudinal halves of sweet potatoes which have been stored for a time are likely to show a greater dissimilarity in composition than the halves of freshly dug potatoes. The differences, however, are not sufficiently great to overshadow the significant differences seen in the later tables. The inequality in composition of the halves of the same potato is much less than the unlikeness of different potatoes. The method of comparison of halves is therefore more satisfactory than the comparison of different whole potatoes unless a sufficient number be used to obliterate, to a great extent at least, errors due to individual differences.

The question whether the cut halves behave in the same way in storage as whole sweet potatoes can be more easily discussed in connection with the data presented later. It should be mentioned here, however, that in the first experiment at 15.5° C. the halves lost an unusual quantity of moisture and that this drying may have had some influence on their behavior. In subsequent experiments precautions were taken to avoid a loss of moisture as far as possible.

EXPERIMENTAL DATA

The data relating to all the experiments are collected in Tables III, IV, and V. Table III contains the data of the three experiments conducted at 30°, Table IV those of the experiments at 5°, and Table V those of the experiments at 15.5° C. Under each experiment the first section refers to the changes in composition of the sweet potatoes during the first period of 10 to 12 days immediately after the roots were dug, while the second section gives the changes during a period of equal length immediately following. The change during each period is shown by the difference in composition between the "a" halves analyzed at the beginning of their respective periods and the "b" halves of the same potatoes analyzed at the ends of the periods. The data in each case are based on the water content of the first half of the potato analyzed. The columns of differences show, respectively, the difference in the percentage of

reducing sugar, cane sugar, and starch in the half of the potato analyzed at the beginning, and the corresponding half analyzed at the end of the same period. These differences therefore represent the increments in the percentage of these substances in the stored halves during the 10- or 12-day storage period.

In the discussion of these tables it will be most convenient to compare the results of the experiments conducted at 30° with those of the experiments conducted at 5° and to consider later the experiments at 15.5° C.

The first and second experiments carried out at 30° (Table III) are similar in plan and execution and the results are entirely congruous, so that they may be discussed together. In both of these experiments there is a marked loss of starch during the first period of 12 days following the digging of the potatoes, but very little further loss during the second period. The changes in cane sugar correspond inversely to the changes in starch. During the first period there is a large increase in cane sugar, but during the second period there is almost no further gain. The figures showing the changes in reducing sugar during the first period are irregular, but during the second period there is a consistent and well-marked loss.

TABLE III.—*Changes in composition of sweet potatoes at 30° C.*

FIRST EXPERIMENT (FIRST PERIOD)

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Difference.	Cane sugar.	Difference.	Starch.	Difference.
	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>		<i>Per cent.</i>	
4a . . .	74.02	0.47	0.37	2.63	1.95	18.91	-3.32
4b . . .	68.63	.84		4.58		15.59	
5a . . .	73.11	.79	.23	2.38	1.68	19.44	-2.42
5b . . .	70.41	1.02		4.06		17.02	
6a . . .	73.73	.91	-.26	2.09	2.43	19.16	-3.05
6b . . .	66.38	.65		4.52		16.11	
7a . . .	73.91	.80	-.06	2.22	2.63	18.88	-3.26
7b . . .	65.71	.74		4.85		15.62	
8a . . .	73.12	.81	.12	2.52	1.30	19.36	-1.93
8b . . .	69.70	.93		3.82		17.43	
9a . . .	74.06	.93	-.32	1.81	2.74	18.66	-3.06
9b . . .	65.26	.61		4.55		15.60	

FIRST EXPERIMENT (SECOND PERIOD)

13a . .	71.10	1.41	-0.89	3.62	0.53	18.84	+0.13
13b . .	64.10	.52		4.15		18.97	
14a . .	72.52	1.23	-.74	3.61	.87	17.93	-.37
14b . .	66.50	.49		4.48		17.56	
15a . .	71.58	1.21	-.81	3.86	.79	18.23	-.09
15b . .	65.01	.40		4.65		18.14	
20a . .	74.15	1.38	-.72	3.79	.74	15.63	+.33
20b . .	66.14	.66		4.53		15.96	
21a . .	72.57	1.24	-.81	4.00	.88	17.49	-.52
21b . .	63.55	.43		4.88		16.97	
23a . .	73.25	1.44	-.86	3.36	.08	17.05	.75
23b . .	66.78	.58		3.44		17.80	

TABLE III.—Changes in composition of sweet potatoes at 30° C.—Continued

SECOND EXPERIMENT (FIRST PERIOD)							
No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Difference.	Cane sugar.	Difference.	Starch.	Difference.
	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>		<i>Per cent.</i>	
61a...	76.39	0.35	0.12	1.71	1.76	16.09	-1.91
61b...	72.21	.47		3.47		14.18	
62a...	73.35	.89		1.93		19.35	
62b...	71.63	.88	- .01	3.83	1.90	17.46	-1.89
63a...	73.12	.71	.12	2.26	1.94	19.42	-2.22
63b...	68.86	.83		4.20		17.20	
79a...	72.48	.73		2.43		20.19	
79b...	69.48	.67	- .06	5.24	2.81	16.99	-3.20
80a...	73.88	.78	- .25	2.64	1.54	18.60	-1.41
80b...	66.98	.53		4.18		17.19	
81a...	74.20	.69		2.42		18.15	
81b...	66.21	.55	- .14	4.60	2.18	15.66	-2.49
SECOND EXPERIMENT (SECOND PERIOD)							
75a...	70.74	0.94	-0.31	3.73	0.75	20.11	-0.76
75b...	70.43	.63		4.48		19.35	
76a...	73.67	1.26		3.74		16.87	
76b...	71.91	.89	- .37	4.32	.58	16.88	.01
77a...	74.19	.90	- .14	4.05	.72	16.26	- .74
77b...	73.69	.76		4.77		15.52	
97a...	73.58	1.17		3.63		17.25	
97b...	72.80	.84	- .33	4.99	1.36	16.14	-1.11
98a...	73.67	1.10	- .18	3.83	1.28	16.93	- .99
98b...	72.65	.92		5.11		15.94	
99a...	74.20	1.23		3.81		16.16	
99b...	71.44	.85	- .38	4.32	.51	16.10	- .06
THIRD EXPERIMENT (FIRST PERIOD)							
100a...	74.78	0.86	0.02	2.76	1.25	17.15	-1.40
100b...	72.65	.88		4.01		15.75	
101a...	77.68	.56		2.63		14.41	
101b...	76.64	.73	.17	3.69	1.06	12.72	-1.69
102a...	74.43	.82	.11	3.39	1.29	17.41	-1.49
102b...	73.25	.93		4.68		15.92	
103a...	76.38	.75		3.21		15.51	
103b...	75.83	1.01	.26	4.46	1.25	13.70	-1.81
104a...	75.72	.77	.28	2.78	1.07	16.25	-1.38
104b...	74.48	1.05		3.85		14.87	
105a...	75.54	.96		3.27		16.10	
105b...	74.99	1.13	.17	4.04	.77	14.86	-1.24
THIRD EXPERIMENT (SECOND PERIOD)							
109a...	70.69	0.76	-0.23	4.53	0.34	19.10	0.05
109b...	70.31	.53		4.87		19.15	
112a...	74.76	1.61		4.32		14.82	
112b...	74.26	1.06	- .55	4.79	.47	14.84	
113a...	74.17	.94	- .24	4.42	.26	15.87	- .18
113b...	72.95	.70		4.68		15.69	
114a...	75.55	1.54		4.23		14.31	
114b...	74.05	1.13	- .41	4.49	.26	14.44	
115a...	73.42	.74	- .19	3.93	.49	16.69	- .43
115b...	73.55	.55		4.42		16.26	
116a...	74.40	1.28		4.49		15.43	
116b...	73.76	1.02	- .26	4.75	.26	15.33	- .10

In connection with the changes in reducing sugar the effect of cutting on the behavior of the potatoes must be considered. One of the most pronounced effects of wounding plant organs is a stimulation of respiration. The respiration of sweet potatoes is nearly doubled when they are split longitudinally, and the effect, though decreasing, extends over many days. By reason of this increased respiration split potatoes consume a much larger part of their reducing sugar than do whole potatoes. Nevertheless, in spite of this excessive respiration, there was, on the whole, during the first period a slight increase in reducing sugar, which is significant in comparison with the distinct loss during the second period. It appears clear, therefore, that more reducing sugar was formed during the first period than during the second; for during the first period the production of reducing sugar kept pace with its utilization, while during the second period the production was not sufficiently rapid to compensate for the quantity used.

Further evidence that more reducing sugar is formed in the potatoes during the first period than is indicated by the figures in the difference column is furnished by the whole potatoes stored with the first set of halves and split at the end of the first period. The percentage of reducing sugar in these "a" halves of the second period is much greater than in the "b" halves of the first period, with which they are comparable as to time of storage. Unfortunately, there is no such control for the behavior of the halves stored during the second period.

The potatoes used in the third experiment at 30° C. were allowed to remain in the ground for 15 days after the vines had been destroyed. They may therefore be considered to have been in "storage" in the ground during that period. The temperatures during that time, as given by observations of the United States Weather Bureau at Washington, D. C., were as follows:

Date.	Maximum.	Minimum.	Mean.	Date.	Maximum.	Minimum.	Mean.
	° C.	° C.	° C.		° C.	° C.	° C.
Oct. 22.	13. 3	1. 6	7. 8	Oct. 30.	12. 8	5. 6	8. 9
23.	17. 8	1. 6	10. 0	31.	8. 9	1. 6	5. 6
24.	18. 9	13. 3	16. 1	Nov. 1.	11. 1	0	5. 6
25.	19. 4	13. 3	16. 7	2.	12. 8	. 6	6. 7
26.	17. 8	10. 6	14. 4	3.	16. 7	-1. 1	7. 8
27.	20. 6	6. 1	13. 3	4.	17. 2	7. 2	12. 2
28.	22. 8	10. 6	16. 7	5.	14. 4	1. 1	7. 8
29.	17. 8	6. 1	12. 2	6.	15. 6	-1. 1	7. 2

If the cutting of the vines has any effect on the carbohydrate transformations in the roots, the initial changes in these potatoes would have been inaugurated during the period after the vines had been cut and while the roots were still in the ground. However, the changes in these followed the same general course as those in the freshly dug potatoes.

There was a large loss of starch and a great accumulation of sugar during the first period, very little further loss of starch and accumulation of sugar during the second period, and a slight increase in reducing sugar during the first period, with a small loss during the second. But if the data of this experiment are compared with the corresponding data of the first and second experiments, it will be noted that the starch content of the sweet potatoes in the third experiment at the time they were dug is, on the whole, lower than that of the freshly dug potatoes in the first and the second experiments, and the cane sugar is higher, as though a part of the starch had already been converted at the time when the roots were dug. Furthermore, it will be noted that the loss of starch and the increment in cane sugar during the first period are a little less than in the corresponding periods of the first and second experiments. These facts show that as a result of the cutting of the vines the carbohydrate transformations had been initiated in these potatoes while they were still in the ground, but that the changes did not proceed as rapidly at the temperature of the soil as at 30°.

The results of the experiments at 30° C. may be summed up thus: In the freshly dug sweet potatoes whose vines were intact there was a large loss of starch and increase of cane sugar during the first period of 12 days, and very little further change in these substances during the second period. The changes in reducing sugar are obscured by the active respiration induced by high temperature and wounding, but, on the whole, the data show that there was a more extensive formation of reducing sugar during the first period than during the second. The potatoes which had been left in the ground for some time after the vines had been cut showed the same general phases of change, but their starch content was on the whole lower and their sugar content higher at the time of digging, and the rate of starch conversion during the first period was lower than in the potatoes dug while the vines were still intact. These conditions indicate that the carbohydrate transformations had proceeded to some extent in these potatoes after the vines had been cut and while the roots were still in the ground.

If the experiments at 5° C. (Table IV) are now examined, a marked contrast is found between these and the experiments at 30°. In the first two experiments with potatoes whose vines had remained active up to the time of digging, the loss of starch during the first period is much less than at 30°, but the loss continues at approximately the same rate during the second period. With respect to the behavior of the cane sugar the contrast between the potatoes at 30° and those at 5° is equally marked. At 5° there is only an insignificant increase in cane sugar during the first period, but a marked increase during the second. The reverse is true of the reducing sugar. There is a considerable accumulation during the first period and a marked reduction during the second.

TABLE IV.—Changes in the composition of sweet potatoes at 5° C.

FIRST EXPERIMENT (FIRST PERIOD)

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Difference.	Cane sugar.	Difference.	Starch.	Difference.
	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>		<i>Per cent.</i>	
33a...	74.22	0.91	I. 33	1.73	0.45	18.53	-1.89
33b...	72.94	2.24		2.18		16.64	
34a...	72.21	1.23	I. 07	2.14	.35	20.29	-1.82
34b...	72.20	2.30		2.49		18.47	
35a...	71.28	1.27	.80	2.27	.38	20.45	-1.05
35b...	69.22	2.07		2.65		19.40	
36a...	71.02	1.37	.90	1.98	.79	20.92	-1.37
36b...	70.71	2.27		2.77		19.55	
37a...	73.99	1.40	I. 00	2.07	.40	17.65	-1.06
37b...	72.82	2.40		2.47		16.59	
38a...	74.52	1.01	.71	2.18	.56	17.42	-1.16
38b...	72.35	1.72		2.74		16.26	

FIRST EXPERIMENT (SECOND PERIOD)

39a...	72.76	I. 93	-0.34	2.62	I. 89	18.00	-1.52
39b...	70.71	I. 59		4.51		16.48	
40a...	73.46	2.19	- .21	2.88	I. 62	16.94	-1.42
40b...	72.40	1.98		4.50		15.52	
41a...	73.74	2.17	- .12	3.06	I. 66	16.69	-1.65
41b...	72.37	2.05		4.72		15.04	
42a...	72.59	I. 79	- .13	3.18	I. 16	17.76	- .90
42b...	70.86	I. 66		4.34		16.86	
43a...	74.52	2.18	- .32	2.65	I. 89	15.82	-1.45
43b...	73.77	I. 86		4.54		14.37	
44a...	73.35	I. 79	- .18	3.19	I. 95	17.23	-2.17
44b...	72.24	I. 61		5.14		15.06	

SECOND EXPERIMENT (FIRST PERIOD)

82a...	70.99	0.53	2.21	2.03	-0.12	21.26	-2.04
82b...	72.61	2.74		1.91		19.22	
83a...	76.07	I. 14	I. 04	2.06	- .04	16.41	-1.63
83b...	75.93	2.18		2.02		14.78	
84a...	72.38	.78	I. 02	2.25	.48	20.37	-1.75
84b...	72.51	I. 80		2.73		18.62	
85a...	73.49	.71	I. 08	2.08	.21	19.37	-1.23
85b...	72.53	I. 79		2.29		18.14	
86a...	73.47	.82	I. 06	2.52	.28	18.94	-1.25
86b...	72.11	I. 88		2.80		17.69	
87a...	73.99	.86	I. 39	1.61	.34	19.25	-1.67
87b...	72.87	2.25		1.95		17.58	

TABLE IV.—Changes in the composition of sweet potatoes at 5° C.—Continued

SECOND EXPERIMENT (SECOND PERIOD)

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Difference.	Cane sugar.	Difference.	Starch.	Difference.
	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>		<i>Per cent.</i>	
88a...	71.16	1.90	-0.51	3.12	2.33	19.23	-1.61
88b...	69.99	1.39		5.45		17.62	
90a...	72.32	2.06	-.27	2.82	1.99	18.04	-1.42
90b...	71.08	1.79		4.81		16.62	
91a...	73.50	2.40	-.15	2.40	2.46	17.04	-2.55
91b...	74.43	2.25		4.86		14.49	
92a...	74.48	1.67	.25	2.91	2.30	15.72	-2.21
92b...	75.24	1.92		5.21		13.51	
93a...	70.65	2.04	-.18	3.40	1.99	19.48	-1.90
93b...	71.43	1.86		5.39		17.58	
94a...	73.26	2.08	-.20	3.66	1.93	16.97	-1.46
94b...	73.16	1.88		5.59		15.51	

THIRD EXPERIMENT (FIRST PERIOD)

125a..	75.81	0.74	0.57	3.04	1.88	15.77	-2.38
125b..	76.31	1.31		4.92		13.39	
126a..	74.55	.98	.42	3.43	1.44	16.79	-1.96
126b..	74.72	1.40		4.87		14.83	
127a..	74.27	1.19	.25	3.48	1.72	16.89	-2.32
127b..	74.52	1.44		5.20		14.57	
128a..	74.87	1.05	.57	3.01	1.20	16.78	-1.92
128b..	74.80	1.62		4.21		14.86	
129a..	76.38	1.16	.18	3.32	1.18	14.62	-1.78
129b..	76.00	1.34		4.50		12.84	
130a..	72.65	.87	.35	3.62	1.25	18.69	-2.23
130b..	73.69	1.22		4.87		16.46	

THIRD EXPERIMENT (SECOND PERIOD)

131a..	73.95	1.44	-0.19	4.67	2.67	14.67	-2.43
131b..	72.90	1.25		7.34		12.24	
133a..	76.15	1.49	.53	4.36	1.90	13.10	-2.59
133b..	77.19	2.02		6.26		10.51	
134a..	75.28	1.89	.22	4.26	2.12	13.94	-2.69
134b..	76.32	2.11		6.38		11.25	
137a..	73.85	1.58	.09	4.60	1.90	15.71	-2.54
137b..	73.63	1.67		6.50		13.17	
138a..	72.71	1.55	-.11	4.91	2.49	16.26	-2.59
138b..	73.40	1.44		7.40		13.67	
139a..	75.55	1.15	-.31	5.61	1.60	12.96	-1.30
139b..	73.28	.84		7.21		11.66	

In the third experiment at 5°, which was carried out with potatoes that had been left in the ground for some time after the vines had been destroyed, the conversion of starch took place during both periods as in the other two experiments, but in contrast with these the accumulation of cane sugar took place not only in the second but also in the first period. At the same time there was a slight increase in reducing sugar during the first period and scarcely any further increase during the second. A

further fact should be noted—viz, that the starch content of these potatoes at the beginning of the first period is comparable in general with that of the potatoes at the beginning of the second period in the other experiments, while the final starch is much lower than in the other two groups. Similarly, the cane-sugar content at the beginning of the first period is comparable with that of the other groups at the beginning of the second period, but the final cane-sugar content is much higher than in either of those.

Here it is even more evident than in the corresponding experiment at 30° C. that the carbohydrate transformations were well under way at the time when the sweet potatoes were dug and that the data given in Table IV merely show the continuation of the processes which had already been started in the ground.

If the experiments at 5° are now summed up, it is found that whether the potatoes had been dug while the vines were still active or some time after the vines had been destroyed there was a fairly uniform loss of starch during both periods. In the first two experiments only inconsiderable quantities of cane sugar were formed during the first period, but during the second period there was a marked accumulation of cane sugar. In the third experiment the accumulation of cane sugar was marked during both periods. In contrast to the cane sugar, there was a considerable accumulation of reducing sugar during the first period in the first two experiments and a slight loss during the second period. In the third experiment there was little or no accumulation during either period.

The results of the experiments at 15.5° C. (Table V) do not present the same degree of uniformity as those at the other temperatures, but certain definite tendencies are evident. In the first experiment the loss of starch was large during the first period, but during the second the loss was not so great. Correspondingly, there was a considerable quantity of cane sugar formed during the first period and much less during the second. Very little change in the reducing sugar is evident during the first period, but during the second there is a distinct loss. It should be recalled here that the halves used in this experiment lost a large amount of water and that their behavior may have been influenced thereby, for from the work of Lundegårdh¹ it appears that the balance between oil and starch and sugar and starch in seedlings is shifted with changes in moisture content. The behavior of the roots in the second experiment is probably more nearly normal. Here the loss of starch is lower during the first period than at 30°, with no further loss during the second. The accumulation of cane sugar is not as great at first as at 30°, but is distinctly larger than during the second period. The increase in reducing sugar during the first period was comparable to that observed at 5°. During the second period there was a slight loss.

¹ Lundegårdh, Henrik. Einige Bedingungen der Bildung und Auflösung der Stärke. Ein Beitrag zu Theorie des Kohlehydratstoffwechsels. In *Jahrb. Wiss. Bot.*, Bd. 53, Heft 3, p. 421-463. 1914.

TABLE V.—Changes in composition of sweet potatoes at 15.5° C.

FIRST EXPERIMENT (FIRST PERIOD)

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Difference.	Cane sugar.	Difference.	Starch.	Difference.
	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>		<i>Per cent.</i>	
10a...	75.18	0.90	-0.24	2.30	2.75	17.17	-3.07
10b...	63.44	.66		5.05		14.10	
11a...	73.17	1.09	-.06	1.84	2.57	19.49	-2.97
11b...	67.55	1.03		4.41		16.52	
12a...	72.96	.82	0	2.88	3.14	19.08	-3.42
12b...	62.87	.82		6.02		15.66	
30a...	72.77	.75	-.07	2.09	2.69	19.63	-3.68
30b...	62.20	.68		4.78		15.95	
31a...	73.26	.94	-.25	2.01	2.73	19.25	-3.51
31b...	67.32	.69		4.74		15.74	
32a...	74.13	1.02	.04	2.44	2.04	17.96	-2.36
32b...	65.64	1.06		4.48		15.60	

FIRST EXPERIMENT (SECOND PERIOD)

24a...	70.10	1.52	-0.63	4.17	2.52	18.53	-2.06
24b...	66.82	.89		6.69		16.47	
26a...	71.85	1.16	-.58	5.12	.86	16.51	-1.29
26b...	68.08	.58		5.98		15.22	
29a...	71.64	1.48	-.60	3.57	1.94	18.26	-1.06
29b...	66.18	.88		5.51		17.20	
51a...	70.71	1.30	-.41	4.46	2.46	17.99	-1.85
51b...	67.13	.89		6.92		16.14	
52a...	69.12	1.36	-.33	5.02	1.12	19.52	-1.16
52b...	66.61	1.03		6.14		18.36	
53a...	71.51	1.44	-.68	4.52	1.91	17.89	-1.68
53b...	67.74	.76		6.43		16.21	

SECOND EXPERIMENT (FIRST PERIOD)

55a...	74.13	0.65	1.16	2.11	0.91	18.52	-1.78
55b...	73.23	1.81		3.02		16.74	
56a...	73.42	.82	1.08	2.66	.75	18.56	-1.48
56b...	73.18	1.90		3.41		17.08	
57a...	73.14	.85	.85	2.23	.87	19.19	-1.47
57b...	71.95	1.70		3.10		17.72	
58a...	73.88	.61	1.00	2.42	1.00	18.50	-1.89
58b...	73.66	1.61		3.42		16.61	
59a...	75.10	.83	.68	2.56	.91	17.23	-1.46
59b...	73.66	1.51		3.47		15.77	
60a...	75.61	.89	.72	2.13	.58	17.15	-1.18
60b...	73.63	1.61		2.71		15.97	

TABLE V.—Changes in composition of sweet potatoes at 15.5° C.—Continued

SECOND EXPERIMENT (SECOND PERIOD)

No. of sweet potato.	Moisture.	Reducing sugar as glucose.	Difference.	Cane sugar.	Difference.	Starch.	Difference.
	<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>		<i>Per cent.</i>	
64a...	73.29	1.05	0.03	3.30	0.36	17.07	0.10
64b...	72.39	1.08		3.66		17.17	
65a...	72.92	1.50	- .16	2.54	.69	17.91	- .34
65b...	71.50	1.34		3.23		17.57	
66a...	70.53	1.76	- .42	3.69	.57	19.24	.13
66b...	68.75	1.34		4.26		19.37	
67a...	73.34	1.39	- .10	3.31	.49	16.84	- .36
67b...	72.49	1.29		3.80		16.48	
70a...	71.72	1.39	- .06	2.91	.40	18.78	- .35
70b...	70.85	1.33		3.31		18.43	
71a...	73.30	1.55	- .21	3.30	.59	16.97	- .23
71b...	71.72	1.34		3.89		16.74	

THIRD EXPERIMENT (FIRST PERIOD)

106a..	75.20	0.77	0.68	2.81	-0.05	16.35	-0.22
106b..	74.32	1.45		2.76		16.13	
107a..	76.92	.72	.49	3.20	.15	14.95	- .66
107b..	74.43	1.21		3.35		14.29	
108a..	77.38	1.13	.60	2.59	.42	14.65	- .91
108b..	75.58	1.73		3.01		13.74	
122a..	75.60	.93	.55	2.92	.45	16.01	-1.18
122b..	75.48	1.48		3.37		14.83	
123a..	75.75	.48	.50	3.90	.12	15.00	- .72
123b..	72.63	.98		4.02		14.28	
124a..	74.54	.98	.55	3.00	.12	17.33	-1.07
124b..	74.24	1.53		3.12		16.26	

THIRD EXPERIMENT (SECOND PERIOD)

118a..	72.76	1.62	-0.01	2.95	0.25	18.26	-0.77
118b..	72.95	1.61		3.20		17.49	
119a..	74.93	1.61	- .20	3.36	.06	15.73	- .12
119b..	74.48	1.41		3.42		15.61	
120a..	75.16	1.74	- .13	3.56	- .13	15.21	.13
120b..	74.11	1.61		3.43		15.34	
141a..	75.21	2.00	- .17	3.86	- .08	15.08	0
141b..	74.53	1.83		3.78		15.08	
142a..	73.73	1.48	.33	3.54	.64	16.42	-1.33
142b..	75.14	1.81		4.18		15.09	
143a..	75.26	1.68	- .15	3.26	- .04	15.24	.02
143b..	75.57	1.53		3.22		15.26	

In the sweet potatoes which had already undergone a period of "storage" in the ground there was on the whole very little further loss of starch and practically no further accumulation of cane sugar. The reducing sugar shows distinct increase during the first period and a loss during the second.

DISCUSSION OF DATA

If the results of these experiments are considered in a general way, it is found that the rate of starch conversion varies with the temperature. At 30° C. the process is rapid at first, but soon appears to approach a point where no further conversion takes place. At 15.5°, if the second experiment is regarded as typical, the rate of starch hydrolysis is less rapid, but at this temperature also the process seems to approach a state of completion. At 5° the process is distinctly retarded, but it continues without decrease during the period covered by the experiments.

The rate of accumulation of cane sugar also varies with the temperature. At 30° the greater part of the cane sugar is formed during the first 10 to 12 days after the roots have been severed from the vines, but the rate of accumulation diminishes rapidly. At 5° very little cane sugar is produced during the first 10 to 12 days, but subsequently the rate of accumulation is considerably increased, as if there were a lag at first in the formation of cane sugar at this temperature.

The behavior of the reducing sugar is obscured by its utilization in respiration. It is nevertheless evident from the data presented in this paper and in former papers that at 30° C. the production of reducing sugar is sufficiently rapid to provide all that is used in respiration and still permit a considerable accumulation which, under normal conditions, is not far behind that at 5°. At 15.5° (second experiment) and at 5° there is a marked accumulation of reducing sugar at first, but at these temperatures, as well as at 30°, there is very little further accumulation, or even a slight subsequent loss.

The apparent lag at first in the accumulation of cane sugar associated with the marked accumulation of reducing sugar at low temperatures may throw some light on the process of the formation of cane sugar from starch. In the experiments at 5° C. reducing sugar was obviously formed during the first period as a result of the conversion of starch. The disappearance of starch continued at the same rate during the second period. During this period there was, however, no further increase in reducing sugar, but a large increase in cane sugar. Since it is not likely that in the one instance reducing sugar resulted directly from the conversion of starch, and in the other, cane sugar, it may be assumed that the production of reducing sugar went on at a rate corresponding to the loss of starch during both periods and that the excess which was produced during the second period was utilized in the formation of cane sugar. In this connection it is worthy of note that the concentration of reducing sugar always remains comparatively low. Even at low temperatures, at which starch transformation goes on continuously and respiration is reduced to a minimum, the reducing sugar content does not rise above 2 to 2.5 per cent. It appears, therefore, that with the exception of the quantity used for respiration the reducing sugar is transformed into cane sugar as fast as it is formed from starch. Its rate of transformation

would, therefore, be correlated with that of the starch. From these considerations it appears that the hydrolysis of starch in the sweet potato results directly in the formation of reducing sugar, as has been observed in cotyledons and other living plant organs, and that the cane sugar is synthesized from the reducing sugar. Cane sugar is therefore the end product of this series of carbohydrate transformations.

It has sometimes appeared from the extensive accumulation of cane sugar in plant organs at low temperatures that this process went on more rapidly at low than at high temperatures. Such a conclusion would seem to be justified if later phases of the process were compared at different temperatures, as illustrated by the data relating to the second periods of the experiments at 30° and at 5°. These data show that during these periods the loss of starch and the gain in sugar was greater at 5° than at 30°. On the basis of the interpretation given above, however, it is clear that all these reactions conform in general to the Van't Hoff temperature rule regarding chemical reactions. Thus, the rate of conversion of starch is higher at 30° than at 5°, but the reaction obviously approaches an end point which is more rapidly approximated at 30° than at 5°; hence, the reaction slows down more rapidly at 30° than at 5°. It is evident also that the production of cane sugar is more rapid at higher temperatures, and that the reaction, which is prolonged at 5°, nears an end point more quickly at 30°. Hence, if these reactions at different temperatures are compared in their later phases, they will appear to be more rapid at the lower temperature. In the common Irish potato, as well as in some other living plant organs, the series of reactions resulting in the production of cane sugar from starch has been found to be reversible. It is not unlikely that in the sweet potato also the reaction is reversible and that thus the attainment of a final equilibrium between the starch, reducing sugar, and cane sugar is explained. The end point of the reaction or the point of equilibrium is greatly shifted with change of temperature, with the effect that at low temperatures the system permits a greater concentration of sugar than at higher temperatures.

On the basis of these considerations a rational interpretation can be given of the rapid initial carbohydrate transformations, which have been mentioned several times and which it was in part the object of this work to study more fully. The fact that there is a comparatively rapid transformation of starch to cane sugar in sweet potatoes during the first few days after they have been dug and a very much slower transformation subsequently is supported by the data of the experiments conducted at 30° and at 15.5°. At 5°, however, the disappearance of starch continues at about the same rate during both periods, while the rate of accumulation of cane sugar is low at first and higher afterwards. All these facts are explicable by the interpretation given above. We have to do here with processes whose rate depends on the temperature and which at

higher temperatures approach an end point very rapidly, so that we find at first a rapid transformation and after a few days almost a cessation of the processes. At 5° the rates of the reactions are greatly reduced, but the processes continue over a much longer period of time, and the starch conversion and sugar accumulation are much more extensive. At this temperature the course of the reactions becomes clear. The conversion of starch results in the formation of reducing sugar. As the concentration of reducing sugar increases, the rate of formation of cane sugar rises, but at first there is a lag in the production of cane sugar.

There remains to be considered the influence of the vines on the carbohydrate transformations of the sweet potato. From work formerly reported it appears that the conversion of starch to sugar does not take place to any marked extent in the growing potato, and that the inauguration of this process is probably associated with the cessation of the flow of materials from the vines. The data of the third series of experiments confirm this suggestion and show that when the vines are destroyed, even if the roots are left untouched in the ground, the carbohydrate transformations begin. In the third series of experiments carried out with sweet potatoes which were left in the ground for some time after their vines had been cut, there is evidence which has been set forth in the description of the experiments that the carbohydrate transformations were well under way when the potatoes were dug. It is therefore safe to conclude that the activity of the vines inhibits the conversion of starch to sugar in the growing sweet potato.

CONCLUSIONS

From the data given in this paper it appears that in the carbohydrate transformations in stored sweet potatoes starch is first converted to reducing sugar and cane sugar is synthesized from the reducing sugar. The rates of starch hydrolysis and of sugar synthesis in a general way conform to the Van't Hoff temperature rule for rates of chemical reactions. At high temperatures the reactions are rapid at first, but soon become slower and approach an end point. At low temperatures the rates are slower and the end point is so shifted as to permit a greater concentration of sugar. The reactions are continuous.

In the growing sweet potato the concentration of sugar remains comparatively low. The extensive conversion of starch into sugar appears to be inhibited by the activity of the vines. When the vines are destroyed and the flow of materials to the roots is thus interrupted, the carbohydrate transformations characteristic of stored sweet potatoes are begun, even if the roots are left in the ground.

DIURESIS AND MILK FLOW

By H. STEENBOCK,

Assistant Chemist, Agricultural Experiment Station of the University of Wisconsin

INTRODUCTION

In studying the comparative efficiency of the nitrogen of alfalfa hay (*Medicago sativa*) and corn grain (*Zea mays*) for milk production, data were accumulated and published from this laboratory which suggested that alfalfa hay when fed in large amounts often acts as a diuretic and thus depresses the volume of milk flow.¹ While this relation was not found with all the experimental animals (cows), it was, nevertheless, deemed of sufficient importance to merit the study of the influence of specific diuretics on milk flow, as it was barely possible that the diuresis which was produced upon the feeding of alfalfa hay was not in itself responsible for the depression of mammary activity.

In view of the importance which hitherto unknown constituents of diets and rations have lately assumed, it is of the greatest interest to dissect the various factors normally operative in the animal body when feeding any of our ordinary rations. Dairy chemists have spent much time and effort in studying the various factors which influence the secretion of milk and its composition. It seemed not improbable that if any of the well-known diuretics were able to influence milk secretion the means to vary the proportion of individual constituents might also be at hand.

EXPERIMENTS WITH DIURETICS ON GOATS

Two goats in full milk flow were used as the experimental animals. They were individually confined in metabolism cages which made possible the separate quantitative collection of urine and feces. They were fed and milked twice a day, the milk of two consecutive milkings being composited for analysis and measurement of volume. Careful measure of the water consumed and urine voided was recorded. Control of the ration consumed was kept only to the extent that results obtained could not possibly be due to variation in food intake. Goat 1, weighing 95 pounds, was fed daily a ration consisting of 2 pounds of oats (*Avena sativa*), 0.5 pound of June-grass hay (*Poa pratensis*), 60 gm. of air-dried casein, 1 pound of fresh sugar beets (*Beta vulgaris*), and 2 gm. of common salt (sodium chlorid). This provided sufficient energy and a suffi-

¹ Hart, E. B., Humphrey, G. C., Willaman, J. J., and Lamb, A. R. The comparative efficiency for milk production of the nitrogen of alfalfa hay and the corn grain. Preliminary observations on the effect of diuresis on milk secretion. In Jour. Biol. Chem., v. 19, no. 1, p. 127-140. 1914.

ciently narrow nutritive ratio to serve excellently for milk production. Goat 2, weighing 81 pounds, was fed from 1.5 to 2 pounds of oats, 0.5 to 0.75 pound of June-grass hay, and 1 gm. of common salt daily, though the latter was often refused. Great care was taken that any variations in salt intake were not of sufficient moment to influence the character of the results obtained. Data obtained during periods of low consumption or of unusual restlessness of the animal were discarded, as such conditions obviously disturb the milk secretion. Everything possible was done to contribute to the comfort of the experimental animal, in accordance with good dairy practice.

First, it was desired to ascertain if specific diuretics were able at all to influence the volume of milk secreted. At the same time in some instances determinations of the total solids and nitrogen in the milk were made. As it was suggested in the publication referred to that the salts of the alfalfa ration might have been responsible for the diuresis, sodium acetate was the diuretic selected for the first trials. It was given per os to goat 1, at first with her drinking water, but later, as larger amounts were given, as a drench immediately after each milking. During a 4-day period, when there were administered, respectively, 20, 20, 50, and 50 gm. of sodium acetate daily, no diuresis resulted and no change in the milk volume occurred. It was not until the dose was increased to 80 gm. that the milk flow was materially affected, but even here, as seen in Table I, the diuresis was not pronounced.

TABLE I.—*Effect of sodium acetate on milk flow of goat 1*

Date.	Water.	Urine.	Milk.	Solids.	Nitrogen in 5 c. c.	Remarks.
	<i>C. c.</i>	<i>C. c.</i>	<i>C. c.</i>	<i>Per cent.</i>	<i>Mgm.</i>	
Nov. 24.	2,950	1,150	860	15.89	34.7	No additions to ration.
25.	2,000	750	830	16.40	35.7	Do.
26.	3,000	1,250	880	15.84	31.5	Do.
27 ^a	2,700	1,250	610	18.22	33.7	Collection from 80 gm. of sodium acetate.
28 ^b	2,000	1,400	660	17.89	31.5	Collection from 100 gm. of sodium acetate.
29 ^c	1,725	450	925	14.34	30.0	No additions to ration.
30 ^d	2,950	635	930	15.25	31.4	Do.
Dec. 1.	1,670	760	985	15.39	34.0	Do.

^a Small amount of the casein beet mixture not consumed.

^b No casein or beets consumed.

^c No casein or beets given; 12 ounces oats left unconsumed.

^d No casein given.

An increase in percentage of the solids in milk with the decrease in volume is pronounced, while the nitrogen content is unaffected. (See p. 566.) While the indications from the data on the administration of sodium acetate are that the volume of milk flow is decreased with diuresis, yet with the administration of such large amounts of the salt as was found necessary, too severe disturbances of the appetite resulted

to make the data serve their purpose. Furthermore, the urine was so strongly alkaline in reaction that from all appearances of the vulva a marked irritation of the urinary tract had resulted. Obviously sodium acetate was not a good diuretic to use for the solution of the problem at hand. Later some success was obtained with the use of sodium citrate, which with goat 2 upon the administration of 40 gm. in two portions increased the urinary volume from 170 to 550 c. c. and decreased the flow of milk from a volume of 395 to 350 c. c. Its use was not continued.

It was suggested that with the now well-known diuretic properties of the methyl purins, theocin might be a suitable agent. It was administered per os to goat 1 in gelatin capsules in two doses daily during a 9-day period, during which the daily dose was gradually increased to 600 mgm. Inasmuch as the dose for man ordinarily is given at 200 to 400 mgm., it must have been large enough; yet at no time was a diuretic effect noticed. Whether this is due to the difficulty of absorption with the ruminant was not determined, but at any rate during rumination the bitter taste of the regurgitated theocin destroyed the appetite of the animal to such an extent that even if it should have been effective in larger doses its continued administration was out of the question.

Urea was used next and with good results when given in large doses, as shown in Table II.

TABLE II.—*Effect of urea on milk flow of goat 1*

Date.	Water.	Urine.	Milk.	Remarks.
	<i>C. c.</i>	<i>C. c.</i>	<i>C. c.</i>	
Dec. 19.....	2, 000	685	790	No additions to ration.
20.....	2, 000	625	770	Do.
21 ^a	2, 675	1, 925	500	Collection from 50 gm. of urea.
22.....	2, 700	600	850	No additions to ration.
23.....	2, 000	700	930	Do.
24.....	3, 000	2, 060	660	Collection from 30 gm. of urea.
25.....	2, 900	1, 160	855	No additions to ration.
26.....	2, 525	1, 260	780	Do.
27.....	3, 285	1, 825	640	Do.
28.....	2, 450	850	786	Do.
29.....	1, 000	460	700	Do.
30.....	2, 575	500	755	Do.
31.....	1, 925	625	740	Do.

^a Little cascina consumed; its feeding was discontinued from here on.

Urea when given in diuretic doses decreases the volume of milk secreted from 18 to 35 per cent, as seen in Table II. The diuresis in each case is followed by a period of one day in which the daily consumption of water is higher than normal, which suggests that the decreased flow of milk is caused by the withdrawal of body fluids from the mammary gland in an attempt of the animal to free its system of the diuretic. Compensation evidently is not immediately effected by the imbibition of sufficient water, and the body secretions are made to suffer as the result.

An attempt was made to accentuate the effect of the diuretic on the milk flow by keeping the water intake at a level which under normal conditions would be entirely sufficient for the animal, but with the additional requirements during diuresis draw heavily upon the body fluids. Goat 1 was used as the experimental animal. See Table III.

TABLE III.—*Effect of urea with constant level of water intake on milk flow of goat 1*

Date.	Water.	Urine.	Milk.	Solids.	Fat.	Nitrogen in 5 c. c.	Remarks.
	<i>C. c.</i>	<i>C. c.</i>	<i>C. c.</i>	<i>Per cent.</i>	<i>Pr. ct.</i>	<i>Mgm.</i>	
Jan. 26..	2,000	715	780	15.06	5.5	33.9	No additions to ration.
27..	2,000	640	840	15.06	5.4	33.2	Do.
28..	2,000	815	695	16.19	6.7	33.0	Collection from 30 gm. of urea.
29..	2,000	550	860	15.70	6.7	29.6	Do.
30..	2,000	675	900	15.06	5.5	32.8	Do.
31 ^a ..	2,000	260	845	15.99	5.9	33.6	No additions to ration.
Feb. 1..	2,000	855	725	16.18	5.9	35.7	Do.
2..	2,000	350	820	15.08	5.7	35.1	Do.

^a Animal very restless; beets not all consumed.

As seen in Table III, renal activity after the effects of the first day was not sufficient to draw noticeably on the mammary secretion for fluids. At no time when urea was given, even in the above experiment, did the animal show any abnormal desire for water; in fact, the water supply when replenished in the morning was usually left untouched for some time. Yet it is hardly to be questioned that the animal was in great need of water. On the morning of February 2, after the previous day's collection had been made, 25 gm. of urea were given in one dose to determine whether larger quantities of urea were necessary to produce the desired results. This amount of urea, while large, would not furnish any more urea for excretion through the kidneys than 70 gm. of protein, and no untoward effects were expected. Yet five minutes after the urea was given the animal lay down and soon passed into violent convulsions, which terminated fatally in 1 hour and 15 minutes. A morphine hypodermic was of no avail in preventing death. A post-mortem examination gave no clue to the cause of death. Apparently the maximum quantity of urea which could possibly be retained with safety in the circulation had accumulated during the previous period of urea administration. With the sudden flooding of the system with the additional 25 gm. of urea the safety limits were exceeded and death resulted. Immediately previous to the administration of the final dose of urea the animal was ruminating and apparently normal in all respects. The urea used was a Kahlbaum preparation and undoubtedly was free from such other toxic compounds as cyanid or cyanate, as no untoward results followed the subsequent use of urea from the same reagent bottle. It was barely

possible that the previous severe régime of sodium acetate and purin feeding may have injured the kidneys sufficiently to account for the results obtained.

In other trials it was repeatedly demonstrated that the administration of urea upon consecutive days would not continue to influence milk secretion even though diuresis obtained. This is brought out in Table IV.

TABLE IV.—*Effect of repeated urea administration on the milk flow of goat 1*

Date.	Water.	Urine.	Milk.	Solids.	Fat.	Nitrogen in 5 c. c.	Remarks.
	<i>C. c.</i>	<i>C. c.</i>	<i>C. c.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Mgm.</i>	
Jan. 15...	1,850	1,135	880	15.00	5.6	33.5	No additions to ration.
16...	2,500	975	840	14.85	5.0	33.5	Do.
17...	2,850	1,125	840	14.56	5.4	34.3	Do.
18...	3,000	1,685	710	16.55	6.5	35.1	Collection from 30 gm. of urea.
19...	3,550	2,100	850	15.72	6.4	32.3	Do.
20...	4,000	2,410	780	16.12	6.8	32.2	Do.
21...	3,000	1,625	845	15.85	6.15	31.6	Do.
22...	3,000	735	965	15.10	5.7	31.7	No additions to ration.
23...	4,100	345	850	15.07	5.4	33.5	Do.
24...	2,075	525	875	15.00	5.25	33.9	Do.

It is significant that the consumption of water upon repeated administrations of urea increases with the diuresis. Whatever factors may be responsible for the symptoms of increased thirst when urea is given, they do not become operative until the water supply of the body is drawn upon so heavily that milk secretion is reduced. The stimulation of the mechanism for maintaining the concentration of the body fluids normal is then sufficient to cause the animal to imbibe enough water for all its excretory and secretory processes.

In this connection it was of great interest to determine the effect of the administration of sodium chlorid upon milk secretion. Table V gives the data obtained with goat 2.

TABLE V.—*Effect of sodium chlorid on the milk flow of goat 2*

Date.	Water.	Urine.	Milk.	Solids.	Fat.	Nitrogen in 5 c. c.	Remarks.
	<i>C. c.</i>	<i>C. c.</i>	<i>C. c.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Mgm.</i>	
July 19...	1,500	190	410	16.13	6.8	30.4	No additions to ration.
20...	1,050	155	390	16.25	6.5	30.4	Do.
21...	2,800	355	420	15.58	6.2	30.3	Collection from 20 gm. of sodium chlorid.
22...	1,175	355	400	15.91	6.6	30.3	No additions to ration.
23...	1,475	160	375	15.44	6.7	29.2	Do.
24...	2,425	580	410	14.59	5.5	28.8	Collection from 20 gm. of sodium chlorid.
25...	1,050	330	415	14.45	5.7	27.9	No additions to ration.
26...	800	170	395	14.77	5.7	29.0	Do.

While diuresis resulted and more water was lost through the gut, as indicated by a softer consistency of the feces, the volume of milk secreted was not decreased. This is to be explained by the fact that simultaneously with the increased urine flow more than sufficient water was consumed to cover the loss. By stimulation of thirst the excessive concentration of the body fluids was prevented, and the milk flow was not decreased.

*In just what manner the relations between milk flow and urinary secretion with alfalfa hay are brought about is not clear. Whatever agent may be responsible for the diuresis, its action evidently is different from that of urea or sodium chlorid as observed in these studies with the goat.

INFLUENCE OF DIURESIS UPON THE COMPOSITION OF MILK

It will be noticed in Tables I and VI that with decrease of milk volume as caused by diuresis the percentage of total solids is increased. This increase is usually completely accounted for by the increase in fat content. The nitrogen content is not changed.

TABLE VI.—*Effect of diuresis on milk solids of goat 2*

PERIOD 1							
Date.	Water.	Urine.	Milk.	Solids.	Fat.	Nitrogen per 5 c. c.	Remarks.
	C. c.	C. c.	C. c.	Per ct.	Per ct.	Mgm.	
May 21...	1,900	40	740	16.36	6.2	33.0	No additions to ration.
22...	1,800	140	705	16.38	6.4	32.7	Do.
23...	850	350	550	18.67	8.3	35.0	Collection from 20 gm. of urea.
24...	1,700	560	675	16.09	7.9	33.0	No additions to ration.
25...	1,570	100	640	16.89	6.9	33.9	Do.
26...	1,340	80	690	16.11	6.2	33.0	Do.
27...	1,490	350	600	16.83	6.9	33.0	Collection from 20 gm. of urea.
28...	1,300	110	650	16.45	6.8	30.9	No additions to ration.
PERIOD 2							
June 1...	1,580	115	635	15.11	5.2	31.9	No additions to ration.
2...	1,830	140	620	15.30	5.4	30.3	Do.
3...	1,275	90	600	15.34	5.8	30.5	Do.
4...	1,800	380	540	15.91	6.3	30.4	Collection from 20 gm. of urea.
5...	1,125	145	600	15.58	5.9	29.5	No additions to ration.
6...	1,790	50	600	15.28	5.4	29.4	Do.
7...	1,850	365	550	15.86	6.4	29.6	Collection from 20 to 25 gm. of urea.
8...	1,940	220	640	15.31	6.0	27.5	No additions to ration.
9...	1,530	130	645	15.43	6.0	29.2	Do.
10...	1,450	130	655	15.05	5.8	29.8	Do.
11...	1,730	195	620	14.11	5.2	28.8	Do.

The constancy of the nitrogen content of the milk made it impossible that any of the administered urea found its way into the milk, which hypothesis was borne out by direct determination of urea in the milk.

One hundred c. c. of milk were measured off into a 250 c. c. volumetric flask, diluted with 100 c. c. of water, and the proteins removed at boiling temperature by the cautious addition of a 10 per cent solution of acetic acid. Generally about 1 c. c. was required. After cooling, the contents were made up to volume, set aside for 10 minutes, and then filtered through a dry folded filter. One hundred c. c. of the filtrate were pipetted off into an aeration bottle made slightly alkaline to phenolphthalein with a 10 per cent solution of sodium hydroxid and then acidified by the addition, drop by drop, of a 10 per cent solution of monobasic potassium phosphate (KH_2PO_4). After incubation for two hours at 41°C . with 2 c. c. of a 10 per cent solution of urease in the presence of toluol, the ammonia was aspirated into *N/28* hydrochloric acid. Fusel oil was used to prevent foaming. The air current was broken up into fine bubbles in the acid by firmly inserting a small plug of glass wool into the end of the tube dipping into the acid. Later, it was found feasible to make the urea determination without the previous removal of the milk proteins, as the fusel oil was sufficiently active in preventing foaming. A small amount of ammonia was found to be present in milk, but as this is practically negligible, the results are expressed as total ammonia in terms of milligrams of nitrogen per 100 c. c. of milk. (See Table VIII.)

TABLE VIII.—*Effect of the administration of urea to goat 2 on the urea content of milk*

Date.	Urea given.	Nitrogen as NH_3 and urea per 100 c. c. of milk.	Urine.	Milk.	Date.	Urea given.	Nitrogen as NH_3 and urea per 100 c. c. of milk.	Urine.	Milk.
	Gm.	Mgm.	C. c.	C. c.		Gm.	Mgm.	C. c.	C. c.
July 10...	0	13.2	190	500	July 16...	20	11.3	580	445
11...	0	10.5	210	510	17...	20	7.5	360	420
12...	0	13.0	80	480	18...	20	10.2	390	415
13...	0	9.0	315	435	19...	0	7.7	190	410
14...	0	9.1	200	480	20...	0	11.4	155	390
15...	20	11.1	345	375					

The independence of the urea excreted and the urea put out in the mammary secretion strongly suggests that the urea in milk in large part is the result of mammary activity and not the result of a mere diffusion from the circulation.

CONCLUSIONS

(1) Urea administered in a diuretic dose is able to decrease temporarily the flow of milk. Upon repeated administration the increased intake of water which follows the impoverishment of the tissues with respect to

water content balances the draft for water imposed by the diuretic, and the milk secretion comes back to normal.

(2) Sodium chlorid with its diuretic action as well as its laxative effect is unable to depress milk secretion under normal conditions, as it simultaneously calls forth an excessive thirst, which increases the water intake.

(3) With the decreased flow of milk caused by a diuretic the percentage of solids is increased. Fat here is the principal variable.

(4) The mammary gland shows no tendency to absorb and subsequently put out in its secretion additional urea absorbed by the circulation.

(5) It is difficult to interpret the results sometimes obtained with alfalfa hay as due to diuresis alone if urea diuresis can be taken as a type.

PETROGRAPHY OF SOME NORTH CAROLINA SOILS AND ITS RELATION TO THEIR FERTILIZER REQUIREMENTS

By J. K. PLUMMER,

Soil Chemist, Division of Agronomy, North Carolina Agricultural Experiment Station

INTRODUCTION

In connection with the detail study of the soils of North Carolina, the writer has had occasion to make many mineralogical analyses of the existing soil types as defined by the United States Bureau of Soils. These examinations have included all types of any prominence thus far encountered in the survey and give some rather interesting data as to the formation and character of these soils which may be of more than local interest.

The available data showing the mineral composition of soils are meager. The scope of those found is so broad that definite conclusions can hardly be drawn as to the relationships which exist between the mineral component and the character of soils. The behavior of the various soil-forming minerals toward the forces of weathering will have to be known before the soil investigator will be able to solve many of the complex problems confronting him.

The methods used in these analyses are essentially those compiled by McCaughey and Frye.¹ Unfortunately, one serious criticism may be made regarding these methods—i. e., the defiance of members of the clay group against identification. It is quite possible that this group plays the most important rôle in the various soil phenomena of all the separates which compose the soil. Yet it would seem that since the clay owes its origin to the coarser particles, some definite knowledge of the composition of the latter would be imperative.

SOILS OF NORTH CAROLINA

The soils of North Carolina are quite heterogenous and furnish well-defined examples for a discussion of the petrography of soils. The State is divided into three provinces, determined largely by the physiographic provinces used in any study of physical geography. There are the old Appalachian, locally known as the Mountain section, Piedmont Plateau, and Atlantic Coastal Plain. As will be shown later, wide variations in the mineralogical composition of the soils of these provinces are encountered.

Practically all of the soils of the mountains are of residual origin and are derived from igneous and metamorphic rocks, mainly gneiss, schists,

¹ McCaughey, W. J., and Fry, W. H. The microscopic determination of soil-forming minerals. U. S. Dept. Agr. Bur. Soils Bul. 91, 100 p., 12 fig., 12 tab. 1913. Bibliography, p. 99-100.

and granites. The sandy loams, sands, and most of the loams are products of the gneiss and granites; the heavier loams, clay loams, and clays have been derived, for the most part, from schists.

With few exceptions, the soils of the Piedmont Plateau are residual. The rocks of this section are varied and complex, being composed of (1) such igneous material as diorite, diabase, gabbro, and granites; (2) such metamorphosed igneous material as gneiss, schists, and slate, and (3) such young sedimentary rocks as Triassic sandstone and shale.

None of the soils of the Atlantic Coastal Plain are residual. They all belong to the broad division known as "transported" and are composed of unconsolidated material laid down from the provinces of higher topography. Because of the abrasive and leaching forces which have entered into their formation, the least resistant minerals have been removed, quartz composing mainly the entire soil mass.

In the mineralogical composition of the soils series here reported, the average analyses of five samples of each series were taken. These samples were selected from widely separated areas in order that the series might be as nearly representative as possible. It was recognized at the outset that it would have been better to show the composition of the various types of a series, but space would not permit such procedure. However, it may be said as a general rule that there are no appreciable differences in the occurrence of the minerals in the various types of a series. There are wide variations in the preponderance of different minerals in the types, but usually each series carries the same minerals in all of its types.

To obtain these results, a separation by mechanical analyses of the sand, silt, and clay of each sample was necessary, and the mineral composition of the sand and coarse silt was determined. The clay particles were discarded as being too small for identification. The results are given in Table I, and include the estimation of all the minerals except quartz—the more abundant or characteristic minerals and the less abundant or secondary in quantity present.

A careful study of Table I will show some rather interesting data concerning the mineral component of the sand and silt particles of these soils. One of the most striking points is the wide difference in mineral complexity between soils of the Appalachian Mountains and those of the Piedmont Plateau and the Atlantic Coastal Plain. The soils of the Porters series are the predominating soils of the former province. The Toxaway soils, which are found in the valleys, are of alluvial origin modified by colluvial wash. In these soils there is a more decided occurrence of the original minerals of the parent rock than is found elsewhere.

TABLE I.—*Mineralogical composition of soils of North Carolina*

APPALACHIAN MOUNTAINS

Series.	Depth.	Percentage of minerals not quartz in—		Abundant minerals not quartz in—		Less abundant minerals in—		Remarks.
		Sand.	Silt.	Sand.	Silt.	Sand.	Silt.	
Porters (surface soil).	<i>Inches.</i> 0 to 8	52	60	Muscovite, biotite, epidote, orthoclase.	Biotite, muscovite.	Epidote, microcline, hornblende, magnetite, chlorite, tourmaline, rutile, zircon, sillimanite, ser-pentine, pyroxene, plagioclase, apatite.	Orthoclase, chlorite, microcline, epidote, sillimanite, rutile, zircon, hornblende, magnetite.	Soil characterized by high content of minerals not quartz, of which biotite and muscovite compose about 20 per cent.
Porters (subsoil)...	8 to 30	57	66	Biotite, muscovite, orthoclase, epidote.do.....	Microcline, hornblende, magnetite, tourmaline, rutile, zircon, serpentine, pyroxene, plagioclase, apatite.	Hornblende, magnetite, chlorite, rutile, zircon, plagioclase, apatite.	As in the soil, the mica content is very high. Biotite is principally fresh; this also applies to orthoclase. Quartz is mainly primary.
Toxaway (surface soil).	0 to 11	50	57	Biotite, orthoclase, epidote, microcline, muscovite.do.....	Hornblende, magnetite, chlorite, sillimanite, tourmaline, pyroxene, apatite.	Chlorite, magnetite, rutile, zircon, sillimanite, microcline, epidote, tourmaline.	Very high content of potash-bearing minerals. Biotite and orthoclase compose over 30 per cent. Both occur as fresh and altered fragments. Quartz grains somewhat rounded.
Toxaway (subsoil).	11 to 30	44	49	Biotite, orthoclase, epidote.	Biotite (altered and fresh), muscovite.	Hornblende, chlorite, magnetite, sillimanite, rutile, zircon, plagioclase.	Chlorite, magnetite, zircon, sillimanite, microcline, epidote, tourmaline.	High content of micas. Biotite at times shows deep-seated chemical alteration. Some secondary quartz present.

TABLE I.—*Mineralogical composition of soils of North Carolina—Continued*
 PIEDMONT PLATEAU

Series.	Depth.	Percentage of minerals not quartz in—		Abundant minerals not quartz in—		Less abundant minerals in—		Remarks.
		Sand.	Silt.	Sand.	Silt.	Sand.	Silt.	
Cecil (surface soil)....	<i>Inches.</i> 0 to 8	30	34	Orthoclase, muscovite, biotite (altered and fresh), epidote, microcline.	Muscovite, biotite, orthoclase, epidote, microcline.	Hornblende, magnetite, rutile, zircon, chlorite, sillimanite, serpentine, garnet, plagioclase, apatite.	Hornblende, chlorite, sillimanite, rutile, zircon, magnetite, garnet, included in quartz.	Rather high content of minerals not quartz. Alteration has taken place among some of the minerals. Plagioclase and apatite found only as traces. Much secondary quartz.
Cecil (subsoil).....	8 to 36	28	33	Orthoclase, muscovite, biotite, epidote.	Muscovite, orthoclase, epidote.	Hornblende, chlorite, magnetite, zircon, rutile, sillimanite, garnet, serpentine, biotite, orthoclase, microcline, plagioclase, apatite.	Hornblende, chlorite, sillimanite, rutile, zircon, magnetite, plagioclase, apatite included in quartz.	Potash-bearing minerals most abundant. Minerals are for the most part altered. Much quartz-carrying infiltrated from oxid.
Iredell (surface soil).	0 to 7	80	Very high.	Hornblende, epidote, augite, biotite.	Hornblende, epidote, biotite.	Muscovite, chlorite, magnetite, plagioclase, orthoclase, microcline, apatite (free and included in epidote).	Muscovite, chlorite, magnetite, plagioclase, orthoclase, microcline.	Quartz is a subordinate mineral in this series, it being less than 5 per cent in the silt. Potash feldspars occur as mere traces.
Iredell (subsoil)....	7 to 30	84	Very high.	Hornblende, epidote, augite, biotite.	Biotite, hornblende, epidote.	Muscovite, chlorite, magnetite, plagioclase, orthoclase, microcline, apatite (free and included in other minerals).	Chlorite, muscovite, magnetite, plagioclase.	Quartz is even less than in surface soil. Biotite has been altered to a lesser degree than found in the surface. Quartz is mainly primary.
Granville (surface soil).	0 to 10	35	40	Microcline, orthoclase.	Microcline, orthoclase.	Epidote, hornblende, magnetite, zircon, rutile, tourmaline, biotite, orthoclase, sillimanite.	Hornblende, epidote, biotite, magnetite.	Potash feldspars compose the greater part of minerals not quartz. Biotite shows deep-seated chemical alteration.
Granville (subsoil).	10 to 28	30	38do.....	Microcline, orthoclase, muscovite.	Hornblende, epidote, magnetite, rutile, zircon, tourmaline, sillimanite, plagioclase.	Hornblende, epidote, magnetite, rutile, zircon.	Potash feldspars as high as 22 per cent of minerals not quartz. Generally more in the silt than found in the surface. Some secondary quartz.

Georgeville (surface soil).	0 to 6	12	16	Orthoclase.....	Orthoclase, muscovite.	Magnetite, sillimanite, epidote, hornblende, rutil, zircon, biotite, tourmaline, magnetite, microcline.	Epidote, sillimanite, hornblende, rutil, zircon, biotite, tourmaline, magnetite, microcline.	Much quartz-carrying coatings of iron oxid. Minerals are a refractory nature. Biotite and orthoclase are badly altered.
Georgeville (subsoil).	6 to 26	14	16do.....	Orthoclase, microcline, muscovite.	Epidote, sillimanite, hornblende, rutil, zircon, microcline, magnetite, tourmaline.	Epidote, tourmaline, sillimanite, chlorite, biotite, rutil, zircon.	Very much the same mineralogical composition as found in the surface soil.
ATLANTIC COASTAL PLAIN								
Norfolk (surface soil).	0 to 7	2 to 4	6 to 9	None.....	None.....	Orthoclase (residues), microcline.	Epidote, tourmaline, zircon, rutil, magnetite, sillimanite, hornblende, muscovite, biotite, garnet.	Soil characterized by low content of minerals not quartz. Those existing are of a decidedly refractory nature.
Norfolk (subsoil)...	7 to 28	5	9do.....do.....	Orthoclase residues.....	Epidote, tourmaline, zircon, rutil, magnetite, sillimanite, microcline, muscovite, chlorite, garnet, biotite.	Little difference between soil and subsoil in mineralogical complexity.
Portsmouth (surface soil).	0 to 6	1 to 3	4do.....do.....	Microcline, tourmaline, sponge spicules, zircon, rutil, hornblende, magnetite, muscovite, chlorite, garnet.	Sponge spicules, zircon, rutil, magnetite, hornblende, muscovite.	Only the highly refractory minerals and sponge residues present.
Portsmouth (subsoil).	6 to 26	2	5do.....do.....	Sponge spicules, microcline, orthoclase (badly weathered), muscovite, chlorite, magnetite, hornblende, zircon, epidote, tourmaline, garnet.	Weathered orthoclase, epidote, hornblende, rutil, zircon, muscovite, magnetite, sponge spicules.	Very much the same condition as found in the surface soil.
Orangeburg (surface soil).	0 to 8	6	7do.....	Microcline.....	Orthoclase, microcline, epidote, hornblende, magnetite, zircon, rutil, tourmaline, chlorite, muscovite, garnet.	Orthoclase, epidote, hornblende, rutil, zircon, muscovite, garnet.	Orthoclase is badly weathered and hornblende shows signs of passing over into epidote. Much secondary quartz here.
Orangeburg (subsoil).	8 to 26	6	9do.....	Orthoclase (residues), microcline.	Orthoclase, epidote, hornblende, magnetite, zircon, rutil, chlorite, tourmaline, garnet, muscovite.	Hornblende, epidote, magnetite, rutil, zircon, muscovite.	Soil characterized by much weathered orthoclase. Very similar to the Norfolk series.

An average of five samples of soil of the Porters series, including types of different texture, shows that 52 per cent of the minerals in the very fine sand separates comprises other minerals than quartz. The potash-bearing minerals are decidedly the predominating ones. Biotite and muscovite mica have been found among the predominating minerals in all five samples, having an average of 20 per cent of all the minerals except quartz. Orthoclase is very abundant in the soils of this province; it, too, has been found among the abundant minerals in all five samples. Microcline is often encountered, especially among the sand particles; however, it is not found as abundantly as orthoclase. A study of the optical properties of biotite and orthoclase often shows them to be undergoing well-marked chemical alteration, the former being metamorphosed to chlorite and epidote and the latter wearing down, leaving a somewhat skeleton-shaped residue. Plagioclase¹ feldspars are encountered often in the soils of this locality; in many instances they are found as well-preserved fragments, which show clean faces and sharp edges, as though little decomposition had taken place.

Another point that may be worthy of note is the accumulation of micas in the silt separates. Not only is this true for the soils of the Appalachian Mountains, but it is most frequently the case with other soils of the United States. If these minerals are found in a soil to any appreciable extent, they usually occur in the largest quantities among the finer particles. This is readily accounted for from their cleavage and other physical properties, which cause them to be quite susceptible to the forces of weathering. As these minerals are carriers of the element potassium, practical significance may be attached to this fact. As they occur among the finer particles, more surface is exposed to the forces which make the soil solution, thereby causing more of this element to be of service to plant life than when found among particles of coarser texture.

Pyroxene and serpentine are found in more abundance in the Mountain soils than is usually the case with those of the Piedmont and Coastal Plain provinces.

Apatite, the mineral carrying the element phosphorus, is somewhat more common in these soils. It is found both as prismatic apatite and as tiny needles inclosed in other minerals. Fry² has called attention to the persistence of included apatite in soils, which may have some bearing on the availability of this element when so found.

The mineral epidote is often found among the predominating minerals of the soils in all parts of the State. Its persistence is readily explained, as it is a product of the metamorphism of the lesser resistant minerals, biotite and hornblende.

¹ The writer has not attempted to differentiate between the members of the plagioclase group.

² Fry, W. H. The condition of phosphoric acid insoluble in hydrochloric acid. *In Jour. Indus. and Engin. Chem.*, v. 5, no. 8, p. 664-665. 1913.

Tourmaline, sillimanite, rutile, and zircon persist in many soil series; in fact, in very few in this State are they entirely absent. They are extremely resistant in character, which is undoubtedly the cause of their persistence.

The soils of the Cecil series are by far the most predominating of the Piedmont Plateau. Though formed from the same general character of rocks, they differ decidedly in mineral complexity. The quantity of minerals other than quartz in the Porters series is nearly double that of the Cecil series. However, minerals of nearly the same kind are encountered in both. As a general rule, greater decomposition has taken place among the minerals of the Piedmont soils; especially is this true of the silt particles. In many of the clay types of the Cecil soils biotite mica is found in only minute quantities, which would tend to show that it is passing out of existence in these older soils. Plagioclase feldspars and apatite are found only in very minute quantities in the soils of this series. Even the quartz particles appear to have undergone much greater wearing than in the mountains.

This is in accord with the work of Coffey¹ in showing the effect of topography upon the composition of soils. In the mountains the forces of erosion have not allowed the soil mantle to become as well defined as it is in the Piedmont Plateau; consequently, there is greater preponderance of the minerals found in the parent rocks when the superficial covering is removed. This fact is better illustrated in the accompanying reproductions of photomicrographs of representative soils of the two provinces (Pl. LII). Quartz and some of the other minerals are eliminated in these cuts, but the relative number of minerals other than quartz in the two samples is easily discernible.

The Iredell soils are formed from the basic eruptives, mica diorite, gabbro-diorite, and meta-gabbro. Quartz is a subordinate mineral, for in the sand portions of five samples whose averages were taken 80 per cent of other minerals than quartz is found. Among the silt particles quartz amounts to only about 5 per cent of the total minerals. Epidote, hornblende, and augite compose the greater part of the particles of coarser texture, while biotite and pyroxene are found more abundantly in the silt. Very little decomposition had taken place among any of the minerals found in this series; even the plagioclase feldspars, which occur in rather large quantities, do not show signs of serious chemical decomposition. An interesting point is the scarcity of the potash feldspars, orthoclase and microcline. Apatite is found in much larger proportions than in any other soil series in North Carolina, which is in accord with the total chemical analysis. As an average of five samples of the Iredell loam, the phosphoric-acid content is found to be 6,251 pounds per acre

¹Coffey, G. N. A study of the soils of the United States. U. S. Dept. Agr. Bur. Soils Bul. 85, 114 p., map. 1912.

for the first $6\frac{2}{3}$ inches, which is considerably higher than the average for the soils of the State. Field experiments which have been conducted on this series for the past five years indicate that phosphorus is in no way the limiting element in crop production.

The Granville soils, which are found in limited areas in the Piedmont Plateau, are formed from sandstone and shale. These soils are unusually high carriers of potassium, which is supplied mainly as microcline and orthoclase. While some biotite and muscovite are encountered, very little of the potassium must come from this source. It would be interesting to have field data on the requirements of the soils of this series for potassium, for comparison with those of the Mountain province, in which mica predominates.

The Georgeville soils represent those formed from Carolina slate, and the minerals other than quartz are mainly the potash feldspars and those of a highly refractory character. Many of the particles carry an infiltration of iron oxid, which makes identification quite difficult. Much of the orthoclase and biotite is badly altered, while other particles of these minerals are found in an unusually fresh condition, which indicates that an admixture of the material which enters into the formation of this soil has taken place.

The soils of the Atlantic Coastal Plain are characterized by their low content of other minerals than quartz. The Norfolk and Portsmouth series are by far the prevailing soils of this province, and, with few exceptions, no particular mineral other than quartz predominates. It might be said in passing that a few instances occur in which the other minerals than quartz will run higher, but this is unusual.

The average among the sand particles for the Norfolk series will not exceed 5 per cent of minerals other than quartz, of which none predominate. Among the particles the size of silt will be found orthoclase residues, microcline predominating. The less abundant minerals are composed mainly of a heterogeneous mixture of the more refractory minerals found in the provinces of higher topography. A point of interest is the scarcity of the micas in the series; they are encountered often, but the quantity found is usually so small that they can be of little value in maintaining the potash content of the soil solution. Apatite and the plagioclase feldspars are rarely found, as they have passed out of existence during the formation of this soil.

The Portsmouth soils are quite similar to the Norfolk, the only distinctive difference being in the amount of organic matter found in the former. On account of their location, which is usually in submerged or recently drained areas, an accumulation of vegetable matter is encountered. The average content of minerals other than quartz in this series is even lower than that of the Norfolk, being 3 per cent. The persistence of sponge spicules or Rhizopoda casts in this series is rather

interesting. These ham-shaped, isotropic particles are the remains of some form of life that flourished here during the submergence of this land.

In the Orangeburg series occurs a higher content of minerals other than quartz than is found in either the Norfolk or the Portsmouth series, but still the amount is small. The soils of the Orangeburg series resemble the Norfolk in many respects, and the same general minerals are encountered.

The low content of other minerals than quartz in the soils of the Atlantic Coastal Plain is in close agreement with the total chemical analyses of the three plant-food constituents—phosphoric acid, potash, and lime. Many chemical analyses of the soils of this province show the above-named elements of plant growth to be exceedingly low. Not only do there appear to be close relationships existing between the total chemical analyses and their mineralogical complexity here, but in the soils of the entire State. This would suggest that since the petrographic methods have reached so high a state of development they may be used with a fair degree of accuracy for estimating the amounts of the mineral plant-food constituents carried by a soil. On account of the ease of manipulation and the time saved in their use, they lend themselves readily for such purpose; especially is this true in scanning soils for the farmer. The information gained is usually not commensurate with the time and expense involved in making “bulk analyses” of soils for farmers. As a rule, it is not necessary that he know the exact number of pounds of plant food contained in his soil; an approximation will usually suffice. A very close estimate as to the quantity of the elements present may be easily secured with the microscopic methods; even more, the way these elements are held is revealed. If more data were at hand showing the availability of the various mineral elements of plant growth furnished by the different soil-forming minerals, more definite information could be obtained as to the fertilizer requirements of the land with the microscope than by “bulk analyses.”

In a former publication¹ the writer submitted data from which there appeared to be some relationships existing with certain crops between the mineralogical and chemical composition of the soils of this State and their requirements for the inorganic elements found in the usual fertilizer mixture—namely, phosphoric acid, potash, and lime. Additional evidence will be submitted along this line, using the cotton plant as the indicator for measuring the relative densities of the soil solution.

In Table II will be found the average results of seven years' fertilizer treatments with cotton at the Iredell Substation, located upon typical Cecil clay loam.

¹ Plummer, J. K. Relation of the mineralogical and chemical composition to the fertilizer requirements of North Carolina soils. N. C. Agr. Exp. Sta. Tech. Bul. 9, 29 p. 1914.

TABLE II.—Average yield of cotton on fields A, B, and C, with seven years' fertilization at the Iredell Substation

Treatment.	Average yield of seed cotton per acre.			Average increase per acre due to fertilizer.
	Field A (1903, 1904, 1906, and 1909).	Field B (1905 and 1907).	Field C (1908).	
	<i>Pounds.</i>	<i>Pounds.</i>	<i>Pounds.</i>	<i>Pounds.</i>
Nitrogen.....	210. 6	377. 5	505. 0	—11. 7
Phosphoric acid.....	655. 6	897. 5	860. 0	441. 8
Potash.....	301. 3	537. 5	435. 0	85. 4
Nitrogen and phosphoric acid.....	897. 5	727. 5	620. 0	520. 1
Nitrogen and potash.....	348. 8	406. 3	400. 0	96. 5
Phosphoric acid and potash.....	855. 0	959. 8	725. 0	608. 0
Nitrogen, phosphoric acid, and potash..	923. 8	1, 002. 3	1, 070. 0	717. 7
Lime.....	97. 5	160. 0	430. 0	27. 0
Lime, nitrogen, phosphoric acid, and potash.....	728. 8	637. 5	945. 0	573. 5

A glance at Table II will show that phosphoric acid is the limiting or controlling element of plant growth for this soil. An average increase for the seven years' treatment of 441.8 pounds is obtained with phosphoric acid alone, while there was an increase of only 85.4 pounds with potash and no increase at all with nitrogen. Nitrogen added to phosphoric acid produced but a slight increase over the latter constituent alone, while potash added to phosphoric acid produced a somewhat better yield.

Table III shows an 8-year average with cotton at the Experiment Station Farm at Raleigh with typical Cecil sandy loam.

TABLE III.—Average yield of cotton on fertilized fields A and B at the North Carolina Experiment Station Farm, Raleigh

Treatment.	Average yield of seed cotton per acre.		Average increase per acre due to fertilizer.
	Field A (1902, 1903, 1904, 1906, and 1908).	Field B (1905, 1907, and 1909).	
	<i>Pounds.</i>	<i>Pounds.</i>	<i>Pounds.</i>
Nitrogen and phosphoric acid.....	1, 154. 5	768. 2	415. 1
Nitrogen and potash.....	994. 6	437. 7	169. 9
Phosphoric acid and potash.....	1, 126. 0	895. 3	464. 4
Nitrogen, phosphoric acid, and potash.....	1, 130. 8	925. 7	524. 6
Lime.....	619. 5	320. 1	31. 9
Nitrogen, phosphoric acid, potash, and lime..	1, 007. 2	975. 3	572. 7

The 8-year average with cotton given in Table III again shows that phosphoric acid is the controlling element in these fertilizer tests. When potash and nitrogen are used in quantities, as in this experiment, only slight increases in yield are produced. The former constituent gave a slightly greater average than did the latter. The average "bulk analyses" of many samples of soil from these two fields, as well as from Norfolk fine sandy loam, will be found in Table IV.

TABLE IV.—Average quantity of the total plant-food constituents per acre in various types of soil

SURFACE SOIL TO DEPTH OF $6\frac{2}{3}$ INCHES (2,000,000 POUNDS)

Soil type.	Nitrogen (N).	Phosphorus pentoxid (P_2O_5).	Potassium oxid (K_2O).	Calcium oxid (CaO).
	Pounds.	Pounds.	Pounds.	Pounds.
Cecil clay loam.....	1, 141	1, 155	7, 213	4, 656
Cecil sandy loam.....	769	503	2, 994	5, 542
Norfolk fine sandy loam.....	853	953	3, 087	3, 220

SUBSOIL TO DEPTH OF 28 INCHES (8,000,000 POUNDS)

Cecil clay loam.....	2, 378	9, 169	25, 090	19, 933
Cecil sandy loam.....	1, 993	4, 007	19, 073	26, 512
Norfolk fine sandy loam.....	1, 360	1, 573	11, 453	8, 880

A comparison of the yields of cotton on the two fields shows marked similarity in fertilizer requirements though the fields are over 100 miles apart. These soils belong to the same series, though of decidedly different texture, one being a rather heavy clay, the other a medium sandy loam. Unquestionably there are numerous other factors than the amount of plant food carried by the two soils which enter into their productiveness; nevertheless, some relationships exist between this question and their requirements for these fertilizer elements. As shown in Table IV, the phosphoric-acid content of both soils is low; until this element has been added in sufficient quantities there can be no increase yields. Although the nitrogen supply in the two soils is found in about the same proportion as the phosphoric acid, it is evidently changed into a more available form faster than the latter element.

The potash content of the Cecil clay loam is about double that of the Cecil sandy loam, both soils showing that potash is in no way the limiting element. Indeed, it is doubtful whether this element can be applied to the former at a profit. A glance at Table I, which gives the mineral composition of the Cecil series, shows that in the fine sand and silt separates the potash minerals predominate and that biotite mica is found among the abundant minerals in all five samples.

Lime has not given material gains with cotton in either test, owing undoubtedly to the physical condition of this land and the large amount of lime carried by the two soils. As a general rule, the minerals which carry lime in the Piedmont soils are more susceptible to chemical and physical decomposition than those found among the fields of the Atlantic Coastal Plain.

Table V gives the average yield of cotton on Norfolk fine sandy loam at the Edgcombe Substation with seven years' fertilization.

TABLE V.—Average yield of cotton on fields A and B with seven years' fertilization at the Edgcombe Substation

Treatment.	Average yield of seed cotton per acre.		Average increase per acre due to fertilizer.
	Field A (1903, 1904, 1906, and 1908.)	Field B (1905, 1907, and 1909.)	
	Pounds.	Pounds.	Pounds.
Control.....	1, 030	429
Nitrogen and potash.....	1, 215	1, 059	376
Phosphoric acid and potash.....	1, 076	873	217
Nitrogen, phosphoric acid, and potash.....	1, 193	1, 022	348
Nitrogen and phosphoric acid.....	1, 108	717	167
Lime.....	1, 061	510	62
Lime, nitrogen, phosphoric acid, and potash.	1, 441	1, 024	499

Table V gives the results of fertilizer tests which are in marked contrast to those obtained from the Cecil series of the Piedmont Plateau. Fertilizer mixtures carrying potash give the most marked yields; in fact, nitrogen and potash give greater returns than the three fertilizer constituents.

Lime in connection with the three fertilizer elements has produced decided gains. The physical condition of this soil is surely as good as that of the Cecil sandy loam at Raleigh, and the amount carried by the soil is quite sufficient to furnish this constituent as a plant food for a number of years to come. The petrographic examination of the Norfolk soils gives epidote as the only lime-bearing mineral of any consequence. It would seem therefore that lime carried in this form is of doubtful value in performing its functions in the soil.

The amount of potash here is even greater than that found in the sandy loam at Raleigh, yet potash seems to be the limiting element on this field. Weathered orthoclase and microcline furnish practically all the potash supply of this soil, while biotite and muscovite micas are much more abundant in the Cecil series.

Another interesting point brought out in these experiments is in regard to the phosphoric-acid content of the three fields. In the Edge-

combe field the content of phosphoric acid is somewhat less than that of the Cecil clay loam at the Iredell farm, yet in the latter soil phosphorus is the limiting element; but this is not the case in the former, owing doubtless to the way this constituent is held in the two soils. The supply of phosphorus must be stored in the organic form. There is practically no apatite in this Norfolk soil, while it is readily encountered in the residual soils of the Piedmont Plateau, occurring both free and included in quartz and other minerals.

CONCLUSIONS

The results of this and other work on the subject indicate that the following conclusions can be drawn, some of which are undoubtedly applicable to other than North Carolina conditions.

Wide variations in mineralogical composition are found between the soils of the Appalachian Mountains, Piedmont Plateau, and Atlantic Coastal Plain. There is unquestionably a greater supply of minerals which carry the inorganic plant-food constituents in the Mountain soils than are found in either the Piedmont Plateau or the Coastal Plain. Though many of the former soils are derived from the same rocks as those of the Piedmont province, the forces of erosion among those of the mountains cause them to contain minerals more nearly the same as the parent rocks than are found elsewhere.

Definite information is required on the behavior of the various soil-forming minerals to the forces of weathering before positive conclusions can be drawn on the availability of the plant food carried by the different minerals.

The field results with the cotton plant indicate that there are some relationships existing between the mineral component of the soil and the requirements of this plant for the three inorganic fertilizer constituents, phosphoric acid, potash, and lime.

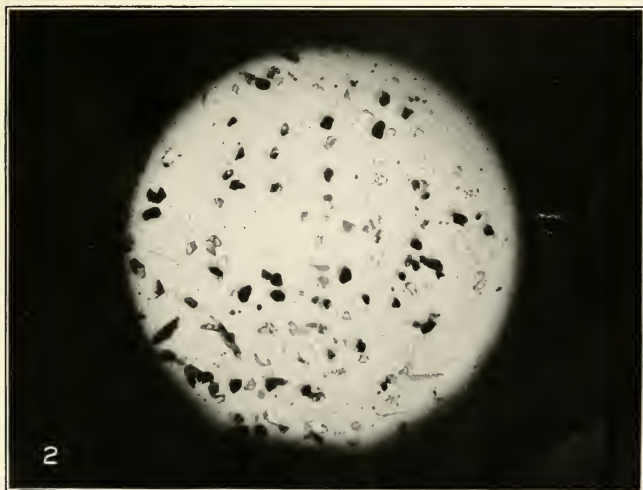
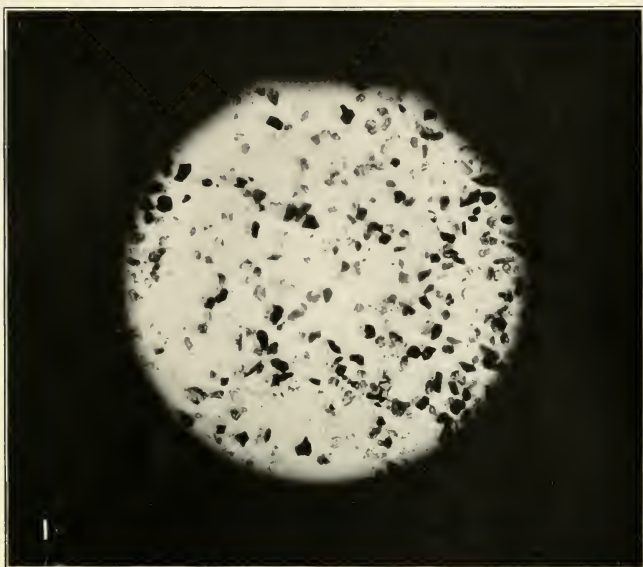
PLATE LII

Fig. 1.—Photomicrograph of Porters soil of the Appalachian, No. 5 sand.

Fig. 2.—Photomicrograph of Cecil soil of the Piedmont Plateau, No. 5 sand.

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